The Pathogenesis of Hidradenitis Suppurativa

By

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Thesis

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Thesis Summary

Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease, the molecular pathogenesis of which is incompletely defined. A number of knowledge gaps in the field of HS exist. These include: an incomplete understanding of molecular disease pathogenesis; the lack of a clear pathogenic paradigm in line with the extant literature; a complete understanding of genetic basis of disease; the identification of factors influencing therapeutic efficacy in HS, and the reasons underlying high placebo response rates in clinical trials. Additionally, there is an urgent need for the identification of novel therapeutic modalities in HS.

The publications included for presentation in this thesis address these six knowledge gaps. Bibliometric and Altimetric evidence supports the importance and high impact of these works to the field. The specific knowledge gaps each group of publications address are as follows:

1) Three publications with novel critical evaluation of the extant literature pertaining to inflammatory cytokines, immunohistochemical studies, as well as mechanisms of action of known therapeutic modalities.

2) Six publications, presenting critical discussion of poorly defined aspects of HS pathogenesis, namely- fibroblasts, complement and B cells, with the development of testable hypotheses about their role in HS. Additionally, novel molecular data regarding the inflammatory characteristics of epithelialized tunnels and inflammatory heterogeneity are presented. These data are integrated into an alternate ‘autoinflammatory’ pathogenic paradigm of disease pathophysiology. This paradigm is

critically evaluated against the current follicular occlusion paradigm and data from the extant literature.

3) Five Publications, Discussing the limited and conflicting evidence surrounding genetic predisposition to disease- and examining the molecular basis of polymorphisms in the gamma secretase complex. Additionally, results of the first genotype-phenotype analysis in HS demonstrates that the current assumptions of the genetics of HS may be incorrect. In-silico analysis of gene expression data in HS suggest that specific gamma secretase substrates other than Notch may play a significant role in disease pathogenesis and is an area which required further research.

4) Three Publications, identifying clinical predictors of clinical response to medical therapies. With regards to the efficacy of current treatment modalities, little is known in order to predict response to therapy. Given that predictive biomarkers in HS are still in their infancy, epidemiological data from recent Phase 3 clinical trials provide novel robust data to demonstrate that epithelialized tunnels, Body Mass Index and family history contribute significantly to the clinical response to Adalimumab across a variety of clinical outcome measures and endpoints.

5) Four Publications, critically evaluating the design and analysis of clinical trials in HS which have led to a number of unsuccessful therapeutics not progressing to the clinic. Important recommendations regarding biopsy definitions for clinical trials, the link between specific outcome measures and elevation in placebo response rates in HS, primary imputation methods in existing trials as well the first analysis of baseline

variability of disease activity in HS which hold great significant for the design of future studies are presented.

6) Two publications describing two open-label clinical trials of Brodalumab in HS. Brodalumab is a novel therapeutic target for disease activity showing high levels of clinical response in these small pilot studies.

In summary, the twenty-three publications presented in this thesis critically evaluate and summarise the extant literature, provide novel evidence regarding the molecular pathogenesis and genetics of disease, identify variables associated with clinical response to current therapeutics, explain the reasons underlying elevated placebo response rates in HS clinical trials and present evidence of a novel therapeutic for use in this disease.

Declaration:

“I, John Walter Frew, certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and
2. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.”

Signed……John Frew……..

Date: 17th January 2022

Acknowledgements:

I would like to acknowledge my mentors, both past and present, who have given me the inspiration and drive to achieve my goals.

I would like to acknowledge my co-authors for all of their hard work, dedication, coffee, ice-cream, bagels, and contributions to this body of work.

I would like to acknowledge my patients. Without their help this work would not be possible. I only hope this work leads to better outcomes and treatments for them.

I would like to acknowledge my family, who keep me grounded. Without their continuous, unconditional love and support none of this would be possible.

“If you put your mind to it, you can accomplish anything”

-Dr E.L. Brown

ABBREVIATIONS:

AD: Atopic Dermatitis

BMI: Body Mass Index

GSC: Gamma Secretase Complex

GWAS: Genome Wide Association Study

HASI: Hidradenitis Area and Severity Index

HiSCR: Hidradenitis Suppurativa Clinical Response

HS: Hidradenitis Suppurativa

IHS4: International Hidradenitis Suppurativa Severity Scoring System

IL: Interleukin

LOCF: Last Observation Carried Forward

ROC: Receiver-Operating Curve

TNF: Tumour Necrosis Factor

CHAPTER 1: INTRODUCTION:

1.1 Hidradenitis Suppurativa: Clinical Presentation

Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease[1] first described by French surgeon Aristide Verneuil in 1854[2]. It manifests clinically as painful deep-seated inflamed lesions, with a predilection for the flexural portions of the skin[1,3]. Whilst it has traditionally been considered a disease of young women[4], HS can affect both men and women of all ages, including children[5]. It typically manifests in areas such as the axillae, inguinal folds and sub-mammary regions although can affect any part of the body[6,7].

The clinical presentations of HS can be varied[8]. The typical presentation involves nodules and abscesses[1,3], however comedones, including pathognomonic double-ended pseudo-comedones can occur[9], as well as hypertrophic scarring, pyoderma-gangrenosum like ulceration and hypertrophic scarring[10]. Additionally, dermal epithelialized tunnels (previous known as sinus tracts) can develop which are chronically painfully and drain a malodorous purulent discharge[11]. Only recently have global consensus definitions of these lesion types been published[10] in order to standardise the description of lesions in HS for use in trials and in the clinic.

Hurley staging is most commonly used to stratify the severity of disease (Figure 1.1.1). Hurley Stage I is defined as solitary or multiple, isolated abscess formation without scarring or sinus tract formation. Hurley Stage II is defined as recurrent abscesses, single or multiple widely separated lesions with/without sinus tract formation. Hurley Stage III is defined as diffuse or broad involvement, with multiple interconnected sinus tracts and abscesses.

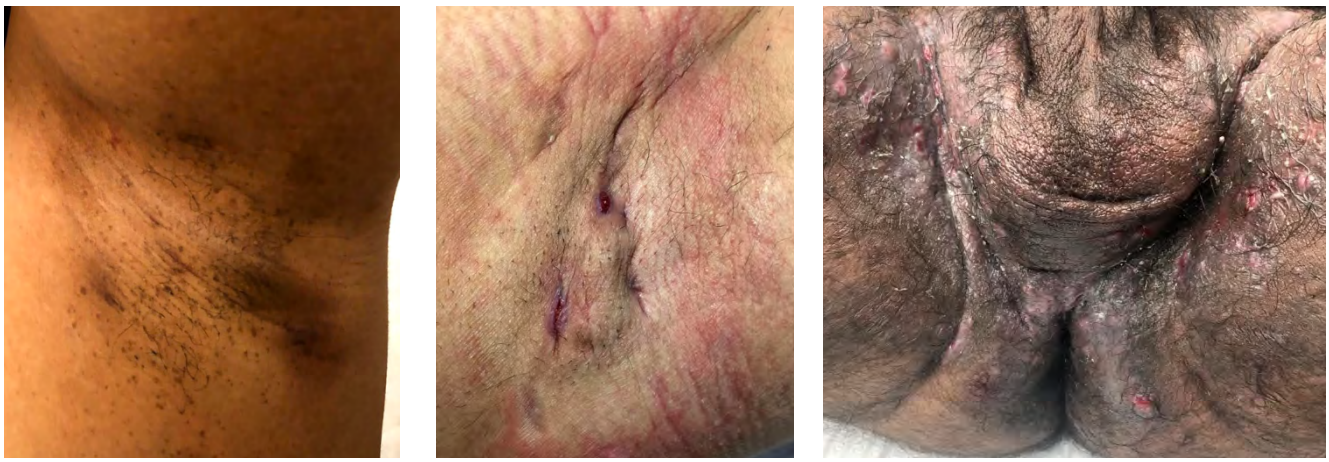

Figure 1.1.1: Hurley Stage I; Hurley Stage II; Hurley Stage III Hidradenitis Suppurativa.

1.2 Demographics of Hidradenitis Suppurativa

The prevalence of HS is estimated at between 0.03%-4% of the population[12] with a significant underdiagnosis and diagnostic delay[13]. The average diagnostic delay from first symptoms to formal diagnosis ranges from 7-10 years[13].

HS has a bimodal age of onset[14], with peaks in late adolescence as well as middle age. Pre-pubescent onset is less common but associated with more severe disease and a family history of HS[5].

A major limitation to understanding the prevalence of HS globally are the sources of current datasets. Most publications are based upon North American or European based cohorts of HS patients, with little large-scale data available from other geographic regions[15]. Many datasets are based upon insurance claims-based data which excludes participants of lower socio-economic status who are unable to access certain forms of health care. Indeed, there is

evidence to suggest that lower socioeconomic populations may have higher rates of HS due to associated factors such as obesity and smoking[16]. Certainly, there is evidence to suggest that the female predilection to HS is not seen in East Asian populations[17]. HS in Asian populations tends to be young male smokers, but few registry-based studies exist to validate this claim.

1.3 Diagnosis of Hidradenitis Suppurativa

Diagnosis of HS is currently clinical and based upon the modified Dessau criteria[18]. The modified Dessau criteria is currently the only widely accepted, validated diagnostic criteria for the disease[18]. (Table 1.3.1) A diagnosis can be made is all three obligatory criteria are present.

Obligatory Diagnostic Criteria for Hidradenitis Suppurativa (Modified Dessau Criteria)
History: Recurrent Painful or Purulent lesions more than twice within 6 months
Location: Groin, Armpit, perimeun, buttock area, submammary/intermammary fold (women)
Primary Lesions: follicular papule/pustule, nodule, abscess (Secondary Lesions: cyst, tunnel*, double pseudocomedone, scar)

Table 1.3.1: Obligatory diagnostic criteria as per the modified Dessau criteria[18].

**NB: in the original text 'tunnel' is referred to (as per the pre-consensus nomenclature) as sinus tract/fistula.*

There is a strong need for the development of diagnostic biomarkers in the disease[19]. Many investigations have identified non-specific markers of cutaneous and systemic inflammation[20,21] however very few studies have examined the validity of any proposed biomarkers. A deeper understanding of the pathogenesis of the disease is needed in order to identify what markers can differentiate HS from other inflammatory disorders in order to develop a consistent diagnostic biomarker to address the issues with diagnostic delay[19].

1.4 Impact of HS upon Quality of Life

HS has a significant impact upon the quality of life of individuals suffering from the disease[22]. It has been reported that the burden of HS is greater than that of many other inflammatory skin diseases including psoriasis vulgaris and atopic dermatitis[23]. Flares from HS can have significant impact upon day-to-day functioning and HS has been documented to have high rates of worker absenteeism[24]. Direct and indirect costs to patients and the health system are significant (>$10,000AUD per patient annually) and vary according to severity of disease[25].

1.5 Current Treatments for Hidradenitis Suppurativa

Treatment for HS is multimodal and needs to consider both the physical cutaneous manifestations of disease, but also the associated comorbidities, psychological and psychosexual impacts of the disease (Figures 1.5.1 and 1.5.2). Medical treatments for HS are varied and include topical, intralesional, antimicrobial, hormonal and immunomodulating therapies[26]. (Figure 1.5.1) Treatments are often targeted based upon the presenting severity of disease, most commonly by Hurley staging. Non-pharmacologic therapies and lifestyle

management including smoking cessation, weight loss and management of inflammatory comorbidities (arthropathy, diabetes, Polycystic Ovarian Syndrome) are important aspects to management of all patients[27-30].

Pharmacological treatments such as topical antiseptics and topical antibiotics are used successfully in Hurley stage 1 disease, with more extensive disease requiring the addition of intralesional corticosteroids, oral antibiotics and intermittent surgical management such as incision and drainage or deroofing of epithelialised tunnels[27-30]. Moderate to severe disease can be managed with combination antibiotic therapy with progression to monoclonal antibody therapy alongside minor surgical procedures[28,31]. Severe (Hurley Stage 3) disease requires multimodal management including monoclonal antibody therapy (most commonly Adalimumab and Infliximab although other agents such as IL-1, IL-17 and IL-23 antagonism are reported[32]), wide excision or laser ablation of recalcitrant disease[31] and management of associated comorbidities.

Management of the associated psychological[33] and psychosexual dysfunction[34] is also an important part of the overall management of the patient, and multidisciplinary management is highly recommended for effective management of the disease.

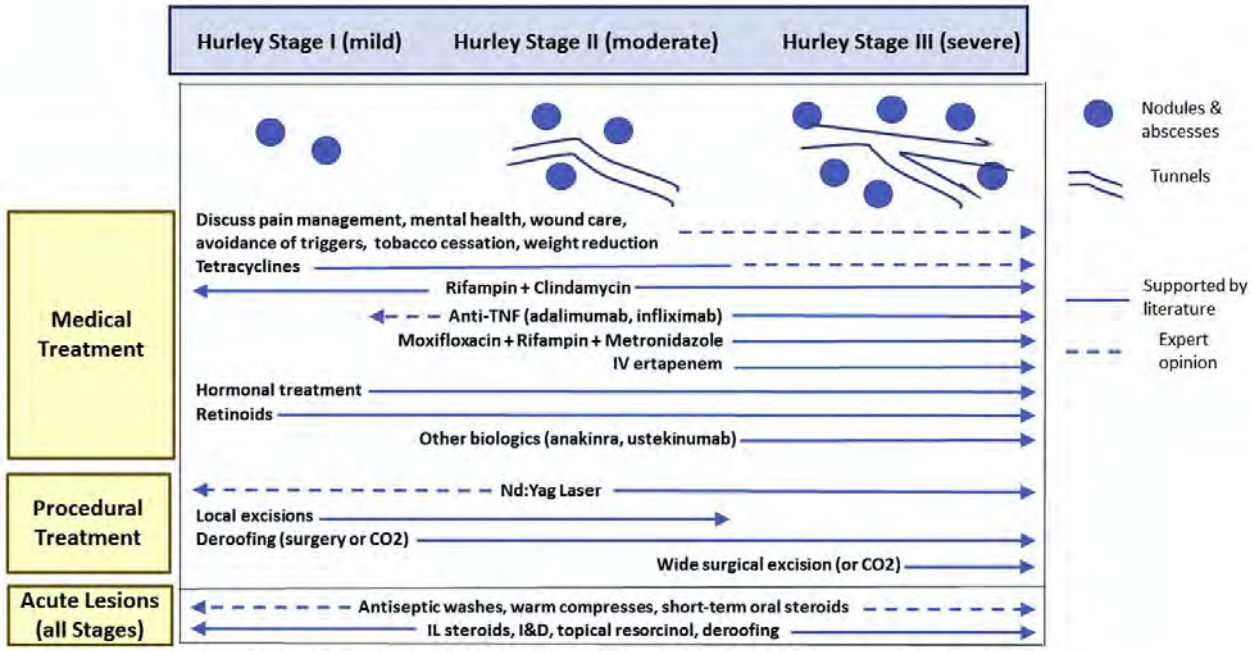

Figure 1.5.1: The range of therapeutic options in the management of Hidradenitis Suppurativa.

(Adapted with permission from Alikhan et al 2019)

Current available therapies often result in incomplete disease control, with current recommendations indicating multiple treatment approaches are often needed to achieve remission[27-30]. A number of therapies used in HS are adopted from other inflammatory disorders such as psoriasis vulgaris. Despite the superficial similarities in epidermal pathology between HS and Psoriasis, there are important differences which however our lack of understanding of disease pathogenesis hampers our ability to identify novel effective therapies for HS for clinical development[19].

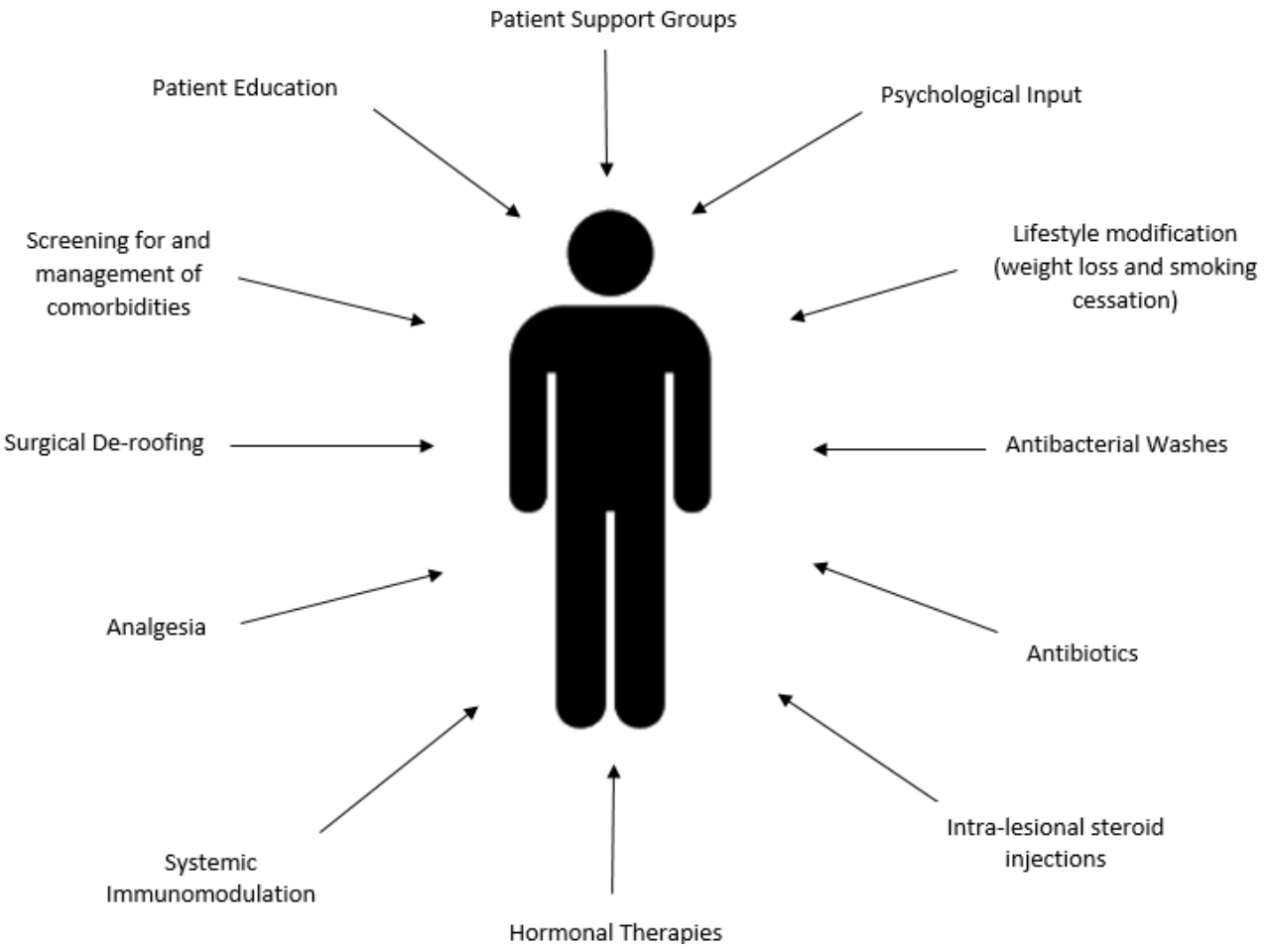

Figure 1.5.2: Multiple Aspects to the management of patients with Hidradenitis Suppurativa.

1.6 Current Knowledge Gaps in HS

There are multiple, significant knowledge gaps in the field of HS which have been previously identified and discussed as priority targets for future mechanistic and clinical research[35]. (Table 1.6.1)

Major Gaps in Understanding and Treatment of Hidradenitis Suppurativa *(Adapted from Hoffman et al 2017[35])*
Diagnosis of HS
Description of HS Clinical Presentations
Natural History of HS
HS Phenotype-Genotype Correlation
HS Clinico-Pathological Correlation
Pathophysiology of HS
Optimal Treatments for HS
HS Treatment Response Outcomes

Table 1.6.1: Significant knowledge gaps in the field of HS (Adapted with permission from Hoffman et al 2017)

A number of these identified knowledge gaps are interlinked. An increased understanding of the pathogenesis of disease would lead to the potential for diagnostic biomarkers, the ability to develop clinic-pathological correlation of disease and identify optimal therapeutics. Additionally, the identified therapeutics can only be adequately tested in randomised, placebo controlled clinical trials if consensus regarding clinical presentations are established and adequately validated outcome measures are established.

1.7 Incomplete Understanding of HS Pathophysiology Hampers Addressing Knowledge Gaps

Our incomplete understanding of disease pathogenesis in HS is the major barrier to developing more reliable screening and diagnostic techniques, to understanding the relationship between genetics and clinical presentations, as well as to identifying and trialling novel therapies[19]. An additional complexity in HS is that disease presentation can be quite heterogeneous[8,9], which has led some authors to speculate that different therapies may be more beneficial to different subsets of patients[36-38]. Investigating and understanding the molecular heterogeneity in the disease is also vital to move forward with the development of more effective therapeutics[19].

CHAPTER 2: AIMS UNDERPINNING THE PUBLICATIONS:

The remainder of this dissertation will present a total of 23 publications which address a number of the knowledge gaps as previously outlined in Chapter 1 (Table 2.1).

Knowledge Gap(s) in HS[35]	Dissertation Chapter
Clinico-Pathological Correlation	Chapter 4.1
Pathophysiology of HS	Chapters 4.1,4.2,4.3,4.6
Phenotype-Genotype Correlation	Chapter 4.3
Optimal Treatments for HS	Chapters 4.4,4.6
HS Treatment Response Outcomes	Chapter 4.5

Table 2.1: Identified Knowledge Gaps In HS and the corresponding dissertation chapters addressing these knowledge gaps.

Firstly, chapter 4.1 will summarise and critically evaluate the extant literature as to the pathogenic mechanisms underlying HS through both systematic reviews of the literature and therapeutic mechanisms of current HS therapies. This will aid in clarification as to the current understanding of disease pathogenesis as well as limitations to current mechanistic investigations.

Chapter 4.2 will then review, discuss and critique the potential role of as-yet underappreciated cell types and immune pathways in HS (fibroblasts, complement and B cells). This will result in novel hypotheses being generated. Additionally, chapter 4.2

presents novel mechanistic data regarding the immunological nature of epithelialised tunnels and the inflammatory heterogeneity of serum proteomics in HS.

Chapter 4.3 presents a review and critical interpretation of identified genetic polymorphisms in HS, providing novel insights into the molecular mechanisms of genetics in HS. Additionally, the first study of genotype-phenotype correlation and in-silico analysis of gamma secretase substrates in HS is included.

Chapter 4.4 identifies clinical factors (including BMI and presence of epithelialized tunnels) associated with clinical response to Adalimumab therapy, as identified in re-analyses of Phase 3 clinical data, are presented. This data has direct clinical relevance to the individuals most likely to respond favourably to TNF-alpha inhibition in the disease.

Chapter 4.5 examines the multiplicity of issues currently facing effective clinical trial design and outcome measures in HS trials. Recommendations on biopsy definitions along with novel data on placebo response rates, primary imputation methods and baseline variability in untreated HS will be presented.

Finally, chapter 4.6 will present the results of two open label clinical trials of an IL-17RA antagonist (Brodalumab) in HS. These studies provide evidence for the benefit of IL-17RA blockade in this disease as a novel therapeutic strategy.

Overall, the publications presented within this dissertation provide a comprehensive review and critique of the existing knowledge gaps regarding the pathophysiology of HS. Additionally, they present novel mechanistic data regarding the disease.

Furthermore, links between the molecular pathophysiology of HS and the clinical manifestations of disease and implications for therapeutics are established. This is achieved primarily through the identification of epithelialised dermal tunnels as an important covariate in clinical response in the setting of current HS therapeutics. Further novel data analysis regarding issues in current HS clinical trial design are then presented with the dissertation concluding with novel clinical data regarding the efficacy of IL-17RA antagonism in a small open-label cohort.

CHAPTER 3: PRESENTED PUBLICATIONS

Included below are synopses of the important and novel aspects of the included publications. Further explanation of the context of these manuscripts and how their novel aspects contribute to the overall understanding of the disease are addressed in Chapter 4. In Chapter 6 article-level bibliometric measures are presented and discussed. This provides objective evidence regarding the exposure and impact of the included publications since their public release.

3.1 Synopses and Documentation of Contributions to Included Publications.

For all of the included publications in this thesis I:

- Conceived or co-conceived the study design
- Developed, wrote and submitted all ethics committee submission applications
- Conducted the recruitment, clinical data collection and biological sample (tissue, blood and serum) samples from all patients
- Conducted or co-conducted the data compilation and analysis
- Lead the manuscript writing and editing for all manuscripts - with the exception of manuscript 2-S2.

Of the 23 publications included in this thesis, 22 are either sole-author, first author or last author publications. In one of the publications (2-S1) the author position is shared as co-first author. In one publication (2-S2) the author position is second-to-last author.

- 3 are sole-author publications
- 18 are first-author publications
- 1 is a last author publication.
- 1 is a second-to-last author publication.

Specific details of authorship and formal documentation of my contributions to each manuscript are presented in below in line with the CRediT contributor role taxonomy[39]. Complete lists of all author contributions (in line with CRediT contributor role taxonomy[39]) are presented in appendix A. Definitions for each of the contributor roles are presented in appendix A.

Publication 1-1: Frew JW, Hawkes JE, Krueger JG "A Systematic Review and Critical Evaluation of Inflammatory Cytokine Associations in Hidradenitis Suppurativa" *F1000Research* (2018); 2018 Dec 13;7:1930

Synopsis: A systematic review critically evaluating the role of inflammatory cytokines in tissue and serum in reported studies of HS. Demonstrates high variability and need for standardized methods.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 1-2: Frew JW, Hawkes JE, Krueger JG "A Systematic Review and Critical Evaluation of Immunohistochemical Associations in Hidradenitis Suppurativa" *F1000Research* (2018); 2018, 7:1923

Synopsis: A systematic review critically evaluating the immunohistochemical findings in studies examining HS. Demonstrates high variability between studies and need for standardized methods.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 1-3: Frew JW, Hawkes JE, Krueger JG "Topical, Systemic and Biologic Therapies in Hidradenitis Suppurativa: Pathogenic Insights Through Examination of Therapeutic Mechanisms" (2018) *Therapeutic Advances in Chronic Disease* 2019; 10: 2040622319830646

Synopsis: A systematic review of the therapeutic mechanisms of reported therapeutics in HS suggesting that therapeutics function through primarily anti-inflammatory mechanisms

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 2-1: Frew JW, Navrazhina K, Maroun M, Lu PJ, Krueger JG Contribution of Fibroblasts to Tunnel Formation and Inflammation in Hidradenitis Suppurativa Exp Dermatol 2019 28(8):886-891

Synopsis: A Viewpoint article exploring the evidence underpinning the role of fibroblasts in HS and associated comorbidities including Pyoderma Gangrenosum and HS-associated Squamous Cell Carcinoma

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 2-2: Grand D, Navrazhina K, Frew JW Integrating Complement into the Molecular Pathogenesis of Hidradenitis Suppurativa Exp Dermatol 2020; 29(1): 86-92

Synopsis: A Viewpoint article exploring the evidence underpinning the role of complement in HS including in associated comorbidities including PCOS and Hyperlipidaemia

Authorship: Last Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 2-3: Frew JW, Grand D, Navrazhina K, Krueger JG "Beyond Antibodies: B-cells in Hidradenitis Suppurativa: Bystanders, Contributors or Therapeutic Targets?" Exp Dermatol 2020; 29(5):509-515

Synopsis: A Viewpoint article exploring the evidence underpinning the role of B cells in the disease including in draining epithelialised tunnels and interactions with neutrophils.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 2-4: Frew JW. Hidradenitis Suppurativa is an Autoinflammatory Keratinization Disease: A Review of the Clinical, Histological and Molecular Evidence: JAAD International 2020;1(1):62-72

Synopsis: A revision of the pathogenic paradigm of the development of HS- positioning it as an autoinflammatory keratinization disease rather than a disease of follicular occlusion.

Authorship: Sole Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 2-S1: Navrazhina K*, Frew JW*, Garcet S, Krueger JG. Epithelialized Tunnels Contribute to Inflammation in Hidradenitis Suppurativa. J Allergy Clin Immunol 2020; doi:10.1016/j.jaci.2020.12.651

Synopsis: Novel data pertaining to the structure and immunological function of epithelialised tunnels in HS. First identification of these structures re-capitulating the cellular structure and immunological function of the overlying epidermis.

Authorship: Co-First Author

CRediT Contribution: Data Curation; Investigation; Visualisation; Writing- Reviewing and Editing

Publication 2-S2: Navrazhina K, Garcet S, Gonzales J, Grand D, Frew JW, Krueger JG In-Depth Analysis of the Hidradenitis Suppurativa Serum Proteome Identified Distinct Inflammatory Subtypes. J Invest Dermatol 2021; doi:10.1016/j.jid.2021.02.742

Synopsis: Novel data pertaining to the heterogeneity in serum proteomic profile of individuals with HS stratified by neutrophilic inflammation with LCN2 as a potential biomarker of disease severity associated with epithelialised tunnels.

Authorship: Second-to-last author

CRediT Contribution: Data Curation; Investigation; Writing- Reviewing and Editing

Publication 3-1: Frew JW, Vekic DA, Woods J, Cains GD (2017) "A Systematic Review and Critical Evaluation of Reported Pathogenic Sequence Variants in Hidradenitis Suppurativa" British Journal of Dermatology 2017; DOI:10.1111/bjd.15441

Synopsis: A systematic review of pathogenic sequence variants associated with HS demonstrating that the impact of documented variants is likely the extracellular compartment questioning the current paradigm of Notch dysregulation in the disease

Authorship: First author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 3-2: Frew JW, Hawkes JE, Sullivan-Whalen M, Gilleaudeau P, Krueger JG "Inter-Relater Reliability of Phenotypes, and Exploratory Genotype- Phenotype Analysis in Inherited Hidradenitis Suppurativa" Br J Dermatol 2019; 2019 Jan 28. doi: 10.1111/bjd.1769

Synopsis: The first Genotype-Phenotype correlation in HS finding no solid link between identified gnotypes and clinical presentation questioning the monogenic nature of HS.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 3-3: Frew JW, Navrazhina K, In-Silico Analysis of Gamma-Secretase-Complex Mutations in Hidradenitis Suppurativa Demonstrates Disease-Specific Substrate Recognition and Cleavage Alterations Front Med 2019; DOI: 10.3389/fmed.2019.00206

Synopsis: In silico analysis of Gamma Secretase Complex associated substrates in tissue RNAseq of HS tissues identifying a number of potential dysregulated substrates which are potential targets for important pathogenic players in HS aside from Notch.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 3-4: Frew JW "We Need to Talk About Notch: Notch Dysregulation as an Epiphenomenon in Inflammatory Skin Disease" (2018) Br J Dermatol 2019 Feb;180(2):431-432

Synopsis: A review of the existing mechanistic literature regarding Notch dysregulation in various inflammatory diseases- proposing that Notch dysregulation is an epiphenomenon associated with keratinocyte proliferation.

Authorship: Sole Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 3-5: Frew JW Navrazhina K No Evidence that Impaired Notch Signalling Differentiates Hidradenitis Suppurativa from other Inflammatory Skin Diseases Br J Dermatol 2020;182(4):1042-1043

Synopsis: An in-silico analysis of Notch dysregulation in various inflammatory skin diseases showing no HS-specific signature of Notch dysregulation in large scale genomic datasets.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 4-1: Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger JG Dermal Tunnels Influence Time to Clinical Response and Family History Influences Time to Loss of Clinical Response in Hidradenitis Suppurativa Patients Treated with Adalimumab. Clin Exp Dermatol 2020; 46(2):306-313

Synopsis: Results of a post-hoc analysis of individual patient data of the placebo arm of two Phase 3 clinical studies (PIONEER 1 and PIONEER 2) demonstrating presence of tunnels and family history are clinical variables associated with time to achieve clinical response.

Authorship: First Author; Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 4-2: Frew JW, Singh N, Jiang CS, Navrazhina K, Vaughan R, Krueger JG. The Impact of Body Mass Index Upon the Efficacy of Adalimumab in Hidradenitis Suppurativa. Front Medicine 2021;8:603281

Synopsis: Results of a post-hoc analysis of individual patient data of the placebo arm of two Phase 3 clinical studies (PIONEER 1 and PIONEER 2) demonstrating that BMI is a confounder in the setting of lower baseline disease activity further clarifying the role of BMI in clinical response.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 4-3: Frew JW, Jiang C, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger J "Malignancy and Infection Risk During Adalimumab Therapy in Hidradenitis Suppurativa" Clin Exp Dermatol 2020; 45(7):859-865

Synopsis: Results of a post-hoc analysis of individual patient data of the placebo arm of two Phase 3 clinical studies (PIONEER 1 and PIONEER 2) indicating that infection and malignancy risk are likely associated with baseline disease rather than treatment.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 5-1: Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR et al Defining Lesional, Perilesional and Unaffected Skin in Hidradenitis Suppurativa: Proposed Recommendations for Clinical Trials and Translational Research Studies Br J Dermatol 2019; 181(6):1339-1341

Synopsis: Consensus Proposals of consistent reproducible definitions of lesional, perilesional and non-lesional tissue for use in future translational investigations in Hidradenitis Suppurativa.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 5-2: Frew JW, Jiang C, Singh N, Grand D, Navrazhina K Vaughan R, Krueger JG Clinical Response Rates, Placebo Response Rates and Significantly Associated Covariates Are Dependent Upon Choice of Outcome Measure in Hidradenitis Suppurativa: A Post-Hoc Analysis of PIONEER 1 and 2 Individual Patient Data J Am Acad Dermatol 2019; 82(5):1150-1157

Synopsis: Results of a post-hoc analysis of individual patient data of the placebo arm of two Phase 3 clinical studies (PIONEER 1 and PIONEER 2) examining how placebo response rates vary across different primary outcome measures (HiSCR vs IHS4) and

associated covariates which influence clinical response between these two outcome measures.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 5-3: Frew JW "Primary Imputation Methods Impact Efficacy Results in Hidradenitis Suppurativa Clinical Trials" J Am Acad Dermatol 2020; 83(2):663-665

Synopsis: Review and Analysis of different primary imputation methods in published clinical trials of biologic therapeutics in HS, demonstrating significant differences when Non-Responder Imputation is used.

Authorship: Sole Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 5-4: Frew JW, Jiang CS, Singh N, Navrazhina K, Vaughan R, Krueger JG Quantifying the Natural Variation in Lesion Counts over time in Untreated Hidradenitis Suppurativa: Implications for Outcome Measures and Trial Design. JAAD International 2020; 1(2):208-221

Synopsis: Results of a post-hoc analysis of individual patient data of the placebo arm of two Phase 3 clinical studies (PIONEER 1 and PIONEER 2) examining the natural variability of lesion counts over a 12-week period.

Authorship: First Author, Corresponding Author

CRediT Contribution: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 6-1: Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, Ungar J, Krueger JG The Effect of Subcutaneous Brodalumab upon Clinical Disease Activity in Hidradenitis Suppurativa: An Open Label Cohort Study. J Am Acad Dermatol 2020; 83(5):1341-1348

Synopsis: Results of an Open-Label Clinical Study of Brodalumab 210mg/1.5mL q2weekly for 24 weeks in Moderate to Severe Hidradenitis Suppurativa.

Authorship: First Author

CRediT Contribution: Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 6-2: Frew JW, Navrazahina K, Garcet S, Sullivan-Whalen M, Gilleaudeau P, Krueger JG Weekly Administration of Brodalumab in Hidradenitis Suppurativa: An Open Label Cohort Study. Br J Dermatol 2020; 184(2):350-352

Synopsis: Results of an Open-label clinical study of Brodalumab 210mg/1.5mL weekly for 24 weeks in Moderate to Severe Hidradenitis Suppurativa

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CHAPTER 4: THE EXISTING FIELD OF KNOWLEDGE AND CONTRIBUTIONS BY SELECTED PUBLICATIONS:

The pathogenic paradigm of Hidradenitis Suppurativa has undergone numerous reiterations and alterations since the first reporting of the condition in 1864[1,2]. Initially considered as an infectious disorder, histological observations in the mid 20th century led to the consideration of HS as a disorder of inflamed apocrine glands[40]. Seminal papers by Shelley and Cahn[40] demonstrated that apocrine follicular epilation followed by belladonna impregnated tape occlusion led to perifollicular inflammation and apocrinitis. Only in the 1990's was it conclusively demonstrated that apocrine gland inflammation was a secondary phenomenon[41] and that the follicular infundibulum is the central site of inflammation in the disease. Melnik's seminal 2013 paper[42] began to shift the pathogenic paradigm of HS away from an apocrine-gland based inflammatory or infectious disorder, to a disorder of follicular occlusion and proposed dysregulated Notch signaling[42,43] as the unifying feature of HS pathogenesis.

Emerging evidence as to the role of the inflammasome[44-47], complement[48,49] and IL-1 isoforms [50-53] has led to the suggestion of HS as an autoinflammatory keratinisation disease[54]. Evidence of systemic inflammation[55,56], activation of B cells[57,58] and plasma cells[57,59,60] have raised the possibility of HS having an autoimmune or antibody-mediated component However, follicular occlusion is still considered the 'primem movens' of HS[1,61] preceding the inflammatory drive of disease.

Until relatively recently, no cohesive pathogenic paradigm supported by mechanistic evidence has existed as to the molecular mechanisms of disease in HS. Investigations into the inflammatory signature of HS in tissue and serum were hampered by mechanistic heterogeneity as well as a lack of defined sampling and analytical techniques. An in-depth critical evaluation of the extant literature from the perspective of mechanisms of investigation was lacking. This was required to identify the most robust methods for analysing the inflammatory nature of the disease and in order to ensure high quality reliable results from future translational investigations.

4.1: Inflammatory Cytokines, Immunohistochemical Studies, and Mechanisms of Current Therapeutics in HS:

Manuscript	Manuscript Reference
1-1	Frew JW, Hawkes JE, Krueger JG "A Systematic Review and Critical Evaluation of Inflammatory Cytokine Associations in Hidradenitis Suppurativa" *F1000Research* (2018); 2018 Dec 13;7:1930
1-2	Frew JW, Hakes JE, Krueger JG "A Systematic Review and Critical Evaluation of Immunohistochemical Associations in Hidradenitis Suppurativa" *F1000Research* (2018); 2018, 7:1923
1-3	Frew JW, Hawkes JE, Krueger JG "Topical, Systemic and Biologic Therapies in Hidradenitis Suppurativa: Pathogenic Insights Through Examination of Therapeutic Mechanisms" (2018) *Therapeutic Advances in Chronic Disease* 2019; 10: 2040622319830646.

Table 4-1-1 Publications Presented in this chapter

Systematic Reviews aim to be unbiased, reproducible, evidence-based collations and critical evaluations of data pertaining to a topic of interest[62]. Often in the setting of randomized clinical trials they may provide meta-analyses of treatment effect to inform clinical decision making. Such reproducible reviews are essential tools in understanding the breadth of evidence and quality of data surrounding a subject[62]. The three publications comprising this first chapter (Table 4-1-1) are systematic reviews pertaining to the pathophysiological aspects of Hidradenitis Suppurativa (Cytokine Associations, Immunohistochemical Associations and Mechanisms of Therapeutics). As mentioned in the introduction, a systematic review and critical evaluation of the literature was a mandatory first step in order to analyse how the molecular evidence can be interpreted in the context of the prevailing pathogenic paradigm of the disease.

Given the incomplete understanding of the pathogenesis of Hidradenitis Suppurativa, an understanding of the inflammatory characteristics of HS tissue is paramount. This involves the identification and acknowledgement of contradictory results between different studies. The influence of specific laboratory methods, tissue sampling techniques and data interpretation upon contradictory results can be discussed, leading to the development of standardized methods for the conduct of clinical and translational trials in HS.

The identification of various different sampling techniques, including the differences in definitions between “lesional”, “perilesional” and “non-lesional” tissue is one major contributor to heterogeneity in inflammatory analysis between studies. Additionally, the variability in control cytokines (cytokines presumed to not be involved in the pathogenesis of disease and hence not alter between involved and uninvolved tissue) such as IL-15, IL-16; as well as high levels of heterogeneity in age, gender, comorbidities and sites of control tissue also play a contributing role to the statistical heterogeneity identified.

The most important acknowledgement is that the central limitation to all observational studies in HS pathogenesis is that the results are only indicative of a single timepoint in the disease process. No studies have accurately assessed the longitudinal changes in inflammatory markers throughout the natural history of untreated disease. The absence of an accurate animal model of HS makes obtaining this knowledge challenging but is essential in order to understand which components of identified inflammation in HS serum and tissues have clinical relevance.

An alternate approach to understand the clinical relevance of inflammatory mediators in HS is to examine the mechanism of action of commonly used and successful therapeutics in the disease. In the third presented publication in this chapter (1-3), a systematic review of published therapeutics in HS was undertaken. Mechanistic data regarding the molecular action of such therapeutics, analysed in a qualitative manner, identify that anti-inflammatory effects are a common theme across the spectrum of therapies reported. Specifically, modulation of the innate immune system is an important contributor to the mode of action of many drugs. It is acknowledged however, that significant variability exists between which therapies work for specific patients. This links in to pre-existing concepts of disease heterogeneity in the disease. Certainly there is a dearth of publications mechanistically examining the heterogeneity of inflammation in HS. This is an important missing link in order to understand the reasons why certain patients respond to specific therapeutic modalities.

4.1.1: Publication 1-1

Frew JW, Hawkes JE, Krueger JG "A Systematic Review and Critical Evaluation of Inflammatory Cytokine Associations in Hidradenitis Suppurativa" F1000Research (2018); 2018 Dec 13;7:1930

SYSTEMATIC REVIEW

A systematic review and critical evaluation of inflammatory cytokine associations in hidradenitis suppurativa [version 1; peer review: 2 approved, 1 approved with reservations]

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Abstract

Background: The pathogenesis of hidradenitis suppurativa (HS) remains unclear. In order to develop effective treatment strategies, a deeper understanding of pathophysiology is needed. This is impaired by multiple small studies with inconsistent methodologies and the impact of co-occurring pro-inflammatory conditions such as smoking and obesity.
Methods: This systematic review aimed to collate all published reports of cytokine studies in tissue, blood, serum and exudate. It was registered with PROSPERO (Registration number CRD42018104664) performed in line with the PRISMA checklist.
Results: 19 studies were identified comprising 564 individual HS patients and 198 control patients examining 81 discrete cytokines. Methodology was highly varied and the quality of studies was generally low. There was a large degree of variance between the measured levels of cytokines. 78.2% of cytokines demonstrated heterogeneity by the chi-squared test for homogeneity and hence meta-analysis was not deemed appropriate. However, a strong and significant IL-17 signalling component was identified.
Conclusions: Cytokines consistently elevated in lesional, peri-lesional and unaffected tissue are identified and discussed. Areas for further investigation include the role of dendritic cells in HS; the contribution of obesity, smoking, diabetes and the microbiome to cytokine profiles in HS; and examining the natural history of this disease through longitudinal measurements of cytokines over time.

Keywords

Hidradenitis Suppurativa, Cytokines, Inflammation, Pathogenesis, IL-17, TNF-alpha

Open Peer Review

Reviewer Status ✓ ✓ ?

	Invited Reviewers		
	1	**2**	**3**
version 1 published 13 Dec 2018	✓ report	✓ report	? report

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Introduction

Hidradenitis Suppurativa (HS) is a chronic inflammatory disease, the exact pathophysiology of which remains poorly defined[1]. Dysregulation of the T_h17: Treg axis[2], IL-36 signalling pathways[3] and keratinocyte-mediated inflammatory cytokines[4] have been demonstrated in lesional skin, blood, serum, and exudate[5–8] although contradictory results exist[4,9]. Given the variable and incomplete response of patients to treatment, including monoclonal antibodies[1], some authors have proposed clinical[10,11], and immunological[5] subtypes of HS in an effort to better predict treatment outcome and response. Thus far, no current schema accurately predicts treatment efficacy.

In order to develop and implement effective treatment strategies in HS, a deeper understanding of the underlying inflammatory pathophysiology is needed. However, due to the heterogeneity of sampling methods, laboratory processing methods and data analysis, comparison across studies is problematic and potentially biased or inaccruate[12]. Heterogeneity of tissue sampling and laboratory techniques alone may explain the inconsistent and conflicting results regarding specific cytokines,[4,9] however, no systematic analysis of cytokine studies has been undertaken to compare results, methodology, and analytical techniques.

An additional complicating factor is that clinical comorbidities, which are strongly associated with disease activity in HS, such as obesity[13], diabetes[14], inflammatory bowel disease[15], and smoking[16], also produce pro-inflammatory cytokines, which affect multiple organ systems including the skin[15,17–19]. Hence, it remains unclear whether the presence or absence of these conditions confound the findings of cytokine studies in HS, and whether clinical stratification of patients is necessary to identify significant pathogenic pathways, which may be amenable to pharmacological intervention. Critical evaluation and analysis of existing studies may also enable meta-analysis, which may identify cytokines, which, in smaller studies, do not have sufficient power to meet statistical significance when compared to controls.

Objectives

The objectives of this systematic review are:

1) To collate and describe all published reports of human cytokine studies in HS including those in skin, blood, serum and exudate.

2) To critically evaluate the sampling, laboratory and analysis techniques used in each study to assess whether comparisons can be made across individual studies.

3) To analyze the heterogeneity of published studies enable meta-analysis

Methods

This systematic review was registered with PROSPERO[20] (Registration number CRD42018104664) and was conducted in line with the PRISMA checklist[21]

Data sources

Information sources for this review included PubMed (1946-July 1 2018), Scopus (2004- July 1 2018) and Web of Science (1990-July 1 2018) as shown in Figure 1. Search strategy is presented in Table 1

Study eligibility criteria

Eligibility criteria for this review included cohort studies, case-control studies and other observational studies with no restrictions of patient age, sex, ethnicity or language of publication. Eligible studies included:

1) Studies reporting the results of cytokine investigations (in cutaneous tissue, serum, blood or exudate) in human subjects clinically diagnosed with hidradenitis suppurativa.

Studies deemed not eligible included those which:

1) Provide no new data but a review or summary of previously published data

2) Provide no comparison with controls or non-lesional tissue

Appraisal and synthesis methods

Data collection was performed independently by 2 authors (JWF & JEH), with any disagreements regarding inclusion of citations being referred to a third author (JGK) for mediation. Information was collected using a standardized data collection form (available as Extended data[22]) with the principal outcomes of interest being the cytokine of interest, measured level of cytokine in lesional HS skin or serum. Comparison data against either peri-lesional, unaffected or control skin or serum was also collated. If data from individual patients was not available then the aggregate data including average change and statistical analyses of the significance of change was collected.

For each individual cytokine, where more than one study reported results, heterogeneity was assessed using the chi-squared tests for homogeneity. Homogeneity was defined as a chi squared value >0.05. All statistical analysis was undertaken using R (version 3.5.1)

Potential sources of bias in the identified studies are acknowledged including the small size of patient cohorts, the variability in sampling, laboratory techniques and the inclusion of patients being treated with a wide-variety of medications including immunosuppressants. Bias was also assessed using the NIH quality assessment tool for observational studies[23].

Results

A total of 367 non-duplicated citations were identified in the literature review (Figure 1). 343 of these articles were removed upon review of titles and abstracts against the pre-defined eligibility criteria. Full text review of the remaining 24 articles excluded

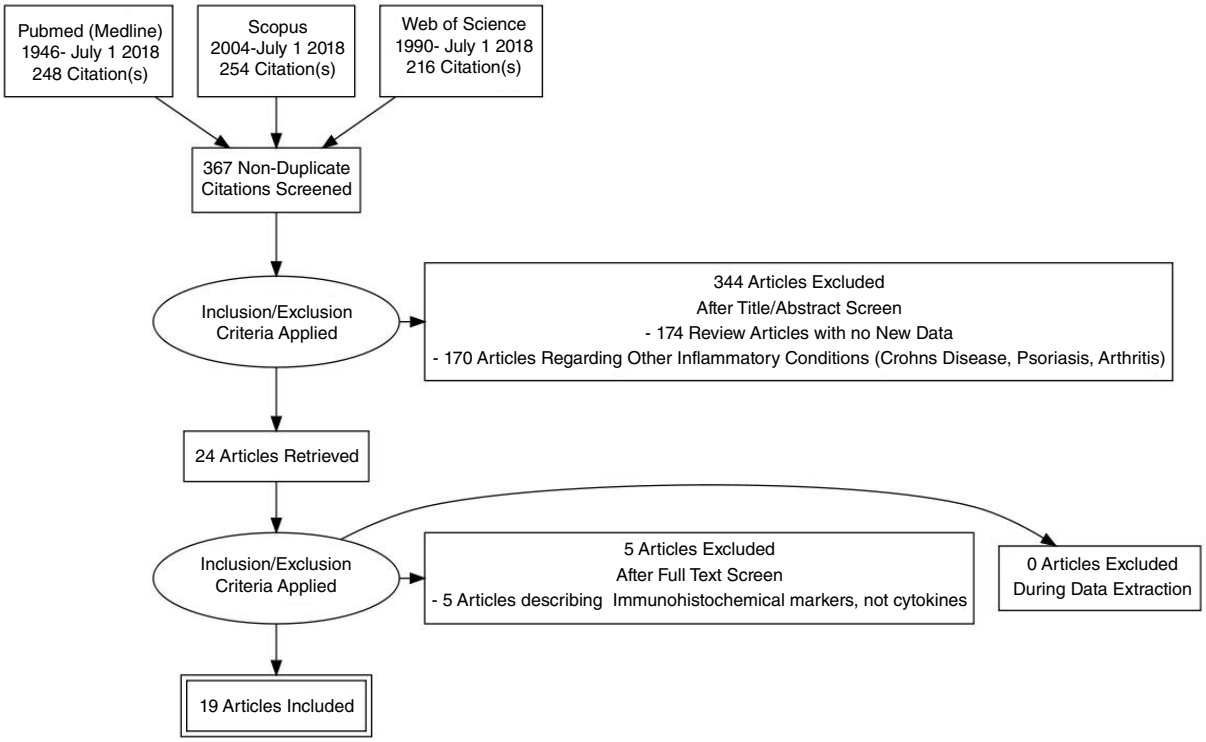

Figure 1. PRISMA Flowchart.

Table 1. Search Strategy.

Resources:
1) Pubmed (1946-July 1 2018), 2) Scopus (2004- July 1 2018) 3) Web of Science (1990-July 1 2018) 4) Published Abstracts 5) Contact with Authors for abstracts without full text for clarification of data and methodology
Pubmed Search Strategy:
acne inversa OR apocrine acne OR apocrinitis OR Fox-den disease OR hidradenitis axillaris OR HS OR pyodermia sinifica fistulans OR Velpeau's disease OR Verneuil's disease OR Hidradenitidis Suppurative AND Cytokine OR chemokine OR inflammatory mediator

5 review articles providing no new data. The remaining 19 studies[2–9,24–33] included the results of 564 individual HS patients and 198 control patients, which were included in this systematic review.

Demographics

The summarized demographic data of the patients and controls comprising this review are included in Table 2. The 564 reported cases comprised of 231 males (40.9% reported cases) and 333 females (59.0%). 24 cases were unreported (4.1%). The average age was 38.5 years (n=560, 18 cases unreported). 141 individuals were current smokers (82.4% reported cases), 8 ex-smokers (4.7% reported cases), 22 non-smokers (12.8% reported cases) and 407 unreported. Obesity (BMI>30) was reported in 85 individuals (42.5% reported cases), with 115 (57.5%) individuals non-obese (BMI<30) and unreported in 378 cases. 8 cases reported diabetes mellitus out of 24 reports (33% of reported cases). 12/38 cases reported a positive family history of HS (31.6% reported cases). Hurley Stage was reported as stage 1 in 68 individuals (17.4% reported), stage 2 in 199 individuals (51% reported cases) and stage 3 in 123 individuals (31.6% reported cases) with 188 cases going unreported. The average mHSS (modified hidradenitis suppurativa score) was 78.1 (n=247 cases). Biopsies were largely taken

Table 2. Demographic data of included studies.

Number of HS Patients	Male	Female	Mean Age (Years)	Comorbidities				Biopsy Sites			Hurley Staging	mHSS Score (Mean)	Therapy	Study Reference
				Smoking	Obesity (BMI>30)	Diabetes	Family History	Axillae	Groin	Genital				
17		1	45	Ex	Y	NR	NR	Serum Measurements			2	NR	Thyroxine	2
		1	39	Y	N	NR	NR				2	NR	N	
		1	24	N	N	NR	NR				2	NR	N	
		1	41	Y	Y	NR	NR				2	NR	N	
		1	23	Ex	Y	NR	NR				1	NR	N	
		1	35	Y	Y	NR	NR				2	NR	N	
		1	30	Y	N	NR	NR				2	NR	Metformin	
		1	41	Y	Y	NR	NR				3	NR	Clindamycin, Rifampicin	
		1	35	Y	Y	NR	NR				3	NR	Metformin	
		1	47	Y	N	NR	NR				3	NR	N	
	1		19	N	N	NR	NR				1	NR	N	
	1		34	Y	N	NR	NR				2	NR	Adalimumab	
		1	47	N	N	NR	NR				3	NR	Adalimumab, Doxycycline	
		1	32	Y	N	NR	NR				2	NR	Adalimumab	
		1	38	Y	N	NR	NR				3	NR	Adalimumab, Doxycycline	
		1	24	Y	Y	NR	NR				2	NR	Adalimumab	
		1	26	E	Y	NR	NR				2	NR	Adalimumab	
18	11	7	(Range 19–62)	NR	NR	NR	NR				NR	NR	NR	24
15	6	9	38.7	NR	NR	NR	NR	N=9	N=4	N=2	Stage 1=0 Stage 2=10 Stage 3=5		N	3

Number of HS Patients	Male	Female	Mean Age (Years)	Comorbidities				Biopsy Sites			Hurley Staging	mHSS Score (Mean)	Therapy	Study Reference
				Smoking	Obesity (BMI>30)	Diabetes	Family History	Axillae	Groin	Genital				
18	1	1	38	N	Y	NR	N	NR	NR	NR	3	54	N	4
	1		42	Y	N	NR	N	NR	NR	NR	3	56	N	
	1		30	N	Y	NR	Y	NR	NR	NR	3	57	Tetracycline	
		1	43	Y	N	NR	N	NR	NR	NR	1	11	Tetracycline	
		1	32	N	Y	NR	Y	NR	NR	NR	1	14	Tetracycline	
		1	14	N	N	NR	N	NR	NR	NR	3	65	Rifampicin, Clindamycin	
		1	47	Y	N	NR	N	NR	NR	NR	3	44	Tetracycline	
		1	43	Y	Y	NR	N	NR	NR	NR	3	22	N	
		1	21	Y	N	NR	N	NR	NR	NR	1	13	Tetracycline	
		1	47	N	N	NR	N	NR	NR	NR	1	11	Tetracycline	
		1	27	Y	N	NR	N	NR	NR	NR	2	7	Tetracycline	
		1	22	N	N	NR	Y	NR	NR	NR	3	68	N	
	1		50	Y	N	NR	Y	NR	NR	NR	2	46	N	
		1	23	N	N	NR	Y	NR	NR	NR	2	22	N	
		1	19	Y	Y	NR	N	NR	NR	NR	2	26	N	
		1	44	Y	N	NR	Y	NR	NR	NR	2	14	N	
	1		22	Y	N	NR	N	NR	NR	NR	3	23	N	
		1	20	N	N	NR	Y	NR	NR	NR	2	21	Tetracycline	
		1	48	Y	N	NR	N		1		3	NR	Rifampicin, Clindamycin	
	1		25	Y	N	NR	N	1			2	NR	Amoxicillin+ Clav Acid	
		1	20	N	N	NR	N	1			2	NR	N	
		1	31	N	Y	NR	N			1	3	NR	Adalimumab	
	1		40	NA	NA	NR	NA				3	NR	N	
		1	46	Y	N	NR	N			1	3	NR	Tetracycline	
		1	26	Y	N	NR	N	1			2	NR	Azithromycin	
		1	36	Y	N	NR	N		1		2	NR	Amoxicillin+ Clav Acid	
		1	29	N	N	NR	y			1	2	NR	Amoxicillin+ Clav Acid	

Number of HS Patients	Male	Female	Mean Age (Years)	Comorbidities				Biopsy Sites			Hurley Staging	mHSS Score (Mean)	Therapy	Study Reference
				Smoking	Obesity (BMI>30)	Diabetes	Family History	Axillae	Groin	Genital				
24	8	16	36.5 (Range 21–51)	NR	NR	NR	NR	NR	NR	NR	Mean=2.29 (SD=0.62)	NR	Untreated	7
74	36	38	37.4 (SD=12.0)	NR	N=32 (43.2%)	NR	NR	Serum Measurements			Stage 1= 11 Stage 2=47 Stage 3=16		All on treatment (Not further elaborated)	8
8	4	4	41.61 (SD=13.81)	N=5 Y=2 Ex=1	NR	N=4	NR	Exudate Measurements			Stage 1=0 Stage 2=3 Stage 3=5	68.88 (SD=41.45)	NR	6
19	11	8	45.6 (SD=10.7)	N=14 (74%)	N=13 (68.4%)	NR	NR	Serum Measurements			Stage 1=0 Stage 2=9 Stage 3=10	82.79 (SD 41.0)	NR	25
19												34.5 (SD 43.5)	Adalimumab	
120	43	77	37.3 (SD=5.9)	NR	NR	NR	NR	Serum Measurements			Stage 1=39 Stage2=52.4 Stage 3=44	28.1 (SD=20.2) 52.4 (SD=24.9) 129.3 (SD=79.2)	NR	5
44	13	31	39.1 (SD=11.4)	Y=34 Ex=4	N=16	NR	NR	NR	NR	NR	Stage 1=5 Stage 2=27 Stage 3=12	NR	N=15 Rifampicin, Clindamycin N=1 Minocycline N=2 Adalimumab n=2 Infliximanb n=24 untreated	31
22	10	12	38.2 (Range 19–60)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	30
3		1	54	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	9
	1		36	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
		1	59	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
10	5	5	42 (Range 21–49)	NR	NR	NR	NR	1	1	N	Stage 2 (100%)	NR	Treatment Withheld	32
20	8	12	37.5 (Range 21–51)	N=18	N=10	NR	NR	NR	NR	NR	NR	NR	Treatment Withheld (8 weeks prior)	29
25	9	16	36 (Range 18–51)	NR	NR	NR	NR	NR	NR	NR	Mean =2.16 (SD=0.55)	NR	Treatment Withheld (3 weeks prior)	28
47	19	28	42.3 (Range 22–54)	NR				Serum Measurements				48.3 (Range 8–144)	NR	27
11	9	2	39.6 (Range 18–61)	NR	NR	NR	NR	NR	NR	NR	"Mod-Severe Disease"	NR	NR	

Number of HS Patients	Male	Female	Mean Age (Years)	Comorbidities				Biopsy Sites			Hurley Staging	mHSS Score (Mean)	Therapy	Study Reference
				Smoking	Obesity (BMI>30)	Diabetes	Family History	Axillae	Groin	Genital				
20	6	14	40 (SD=15)	19	27.6 (4.1)	NR	NR	7	12	1	Stage 1=4 Stage 2=11 Stage 3=5		Treatment withheld 3 weeks prior	26
10	1	9	38 (SD=15)	10	28.9 (SD 4.5)	NR	NR	3	7	0	Stage1=2 Stage2=7 Stage3=1		Treatment Withheld 3 weeks prior	
10	7	3	46.6 (SD=15.1)	10	29.4 (4.7)	3	2	Serum			Stage 3=10	195.6 (SD=97.9)	MABp1	33
10	6	4	49.3 (SD=9.8)	8	27.9 (7.1)	1	2				Stage 2=2 Stage 3=8	124.9 (SD=73.7)	No Treatment	
TOTAL: 564	231	333	38.5	141	85 (0f 200)	8 (of 24)	12	32	35	6	Stage 1= 68 Stage 2=199 Stage 3=123	Average =78.1 (n=247)	Clindamycin+ Rifampicin=18; Adalimumab=26; Metformin=2; Treatment withheld= 85; Thyroxine=1; MABp1=10; Tetracyclines=12; No Treatment=86; Not Specified=74; Infliximab=2; Antibiotics=4; Not Reported=258	

BMI= Body Mass Index mHSS= modified Hidradenitis Suppurativa Score (Sartorius Score) NR= Not Reported SD= Standard Deviation Y= Yes N=No Ex= Ex Smoker

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from the axillae (n=32, 43.8%) and groin (n=35, 48.0%), with a minority of samples being taken from the genital and perianal region (n=6, 8.2%). At the time of sampling patients were on treatment including Clindamycin+ Rifampicin (n=18); adalimumab (n=26); Metformin (n=2); levothyroxine (n=1); MABp1 (n=10); tetracyclines (n=12) Infliximab (n=2); other antibiotics (n=4). Treatment was not specified in 74 cases, with no treatment in 86 individuals and treatment withheld in 85 patients.

Only 5/19 (26.3%) studies analysed both lesional tissue and serum levels of cytokines, enabling direct comparison between these two compartments. 8/19 (42.1%) studies provided age and sex matched controls, 5/15 (33.3%) studies stratified by disease severity and no studies stratified by lesion site or comorbidities. 8/19 (42.1%) studies stratified or accounted for treatment or reported discontinuing treatment up to 3 weeks prior to sample collection (Table 3).

Cytokine analysis

A total of 81 discrete cytokines were analysed over the 19 studies (presented in Table 4). 6 studies provided a total of 78 outcomes from tissue of lesional or peri-lesional biopsies, 4 studies provided a total of 30 results from serum analysis and 1 study provided 15 results from exudate analysis. The remaining 8 studies did not provide quantification of cytokine levels but did provide analysis of the change and significance between lesion and control samples. The degree of change between lesional and control samples varied widely from 1.5 times the control level (IL-1RA p=0.0112) to 149 times the control level (IL-17 p<0.05). 33 cytokines were evaluated in more than one study. Only IL-1β, IL-6, IL-8, IL-17A and TNF-α had data from 5 or more separate studies.

Cytokines and inflammatory proteins which were elevated in more than one study in lesional tissue included IL-1β, IL-6R, IL-10, IL-17A, IL-36α, IL-36β, IL-36γ, IL-36RA, TNF-α, sTNFR2, hBD1, hBD2, hBD3, s100A7, LL37/Cathelicidin, CCL3, CCL5, CCL27 and BLC. Cytokines and inflammatory proteins elevated in peri-lesional tissue included IL-1β, IL-17, IL-36β, IL-36RA, IL-37, IL-38 and TNF-α. IL-37 was the only cytokine identified which showed significant differences between lesional and peri-lesional tissue, with a 1.81 times elevation in lesional compared to peri-lesional tissue (p=0.0002)[3]. IL-17 was elevated in unaffected HS tissue compared to control patient tissue (p<0.05) in one study[31]. In HS tissue, S100A9, hBD1 and hBD2 were reduced but this data did not meet statistical significance. Two studies measuring IL-1β levels showed no statistically significant difference between lesional and control skin[7,25]. No significant elevation of IL-6 was seen in lesional tissue compared to control with the exception of 1 study[25]. IL-8 levels only just made significance in two studies[5,7], with one study showing significant elevation of IL-8 in lesional compared to control tissue[24]. Two additional studies showed no significant difference[4,8]. TNF-α levels were significantly elevated compared to control tissue in two studies[7,31] but not significantly in 2 additional studies[4,24]. sTNFR1 was significantly elevated in one study[26] whilst showing a non-significant difference in a second study[25]. CCL5 was significant in 2 studies in lesional tissue compared with controls[4,26]. One methodology using muramyl dipeptide (MDP) did not reach statistical significance compared to stimulation with Pam2CSK4 Lipopeptide, and non-treated (NT) cells. IFN-γ was elevated in lesional tissue with no significance in one study[28] and significance in another[4].

Elevated cytokines and inflammatory proteins in HS serum included IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17, TNF-α, sTNFR1, CRP, ESR, LC2, and MMP2. TNF-β, and IFN-γ were elevated in wound exudate from active HS lesions. IFN-γ was noted to be decreased in HS patient serum compared to healthy control serum, despite the elevation in wound exudate. Conflicting results were seen in serum findings in IL-10, IL-17 and IFN-γ. One study demonstrated elevated serum IL-10 levels compared to control[5] whereas two other studies[8,27] showed no significant difference. Whilst two studies[4,5] illustrated elevated IL-17 Serum levels in HS patients, one study[7] showed no significant difference between patients and controls. IFN-γ showed no statistically significant decrease in the serum of HS patients compared to control in one study[9] but a significant difference in a larger, higher powered study[4].

Because adalimumab improves HS through TNF antagonism[1,2], this cytokine must be classified as pathogenic. TNF mediates inflammation in a classic "sepsis" cascade in tissues—in this pathway LPS from gram negative bacteria activates TNF release from cells, and then TNF stimulates production of IL-1b, IL-6, and IL-8, leading to neutrophil attraction into sites of infection[2,4]. Increases in IL-1β and IL-8 measured in HS, as well as neutrophil accumulation, could result from this pathway. Alternatively, in psoriasis, TNF is a major cytokine that acts on the IL-23/Type 17 T-cell pathway at two points. First TNF induces IL-23 synthesis in myeloid (CD11c+) dendritic cells in the skin[34]. Second, TNF (as well as other cytokines that also activate NF-kB) act synergistically with IL-17A or IL-17F to increase synthesis of many other cytokines, chemokines, and inflammatory molecules in keratinocytes and other cell types. There are several clues that an IL-23/Type17 T-cell pathway may be active in HS which include detection of T_h17 T-cells in skin infiltrates, increased production of IL-17A, and increased production of LL-37/cathlecidin, S100A7, S100A8, S100A9, LCN2, IL-8, beta-defensins and IL-36; which are all molecules induced by IL-17 in keratinocytes, as also the presence of psoriasis-like epidermal hyperplasia in some reports. The increased production of CCL20[4], would be predicted to increase tissue infiltration of both T_h17 T-cells and CD11c+ DCs, which have both been observed in HS, and increased production of TGF-β could increase differentiation of T_h17 T-cells from precursors and/or influence scarring in skin lesions. If IL-17 is driving inflammation in HS, one would expect to see increased production of additional chemokines that regulate neutrophil chemoattraction (CXCL1, CXCL2, CXCL3). Epidermal hyperplasia is not presently explained in HS, but this could be related potentially to increased expression of IL-19, IL-20 or IL-22, which are associated with the IL-23/Type 17 T-cell axis. If IL-22 is produced in HS lesions, this would implicate T_h22 T-cells as a T-cell type also associated with the IL-23/Type 17

Table 3. Critical evaluation of methodology of studies included in this review.

Cytokines Measured	Number of HS Patients	Number of Controls	Samples Analyzed	Age/Sex Matched Controls	Timing of Samples	Stratified by severity	Stratified by lesion site	Stratified by Co-morbidities	Stratified by Treatment	Sample Storage Time	Sample Types	Study Reference
IL-17 IL-22 IFNg IL-2 IL-10 GM-CSF	17	9	L, PL, U, C, S	Y	NR	NR	N	N	Y	NR	Skin, Serum	2
S100A7 Lysozyme LL37 hBD3 α-MSH MIF TNF-α IL-8 MHC1	18	12	L	N	NR	NR	N	N	N	NR	Skin	24
IL-36α IL-36β IL-36g	15	15	L, PL	NR	NR	NR	N	N	N	NR	Skin	3
IL-17 IL-22 IFNg CCl20 CCL27 S100A7 S100A8 IL-1B CCL5 IP10 IL-8 IL-6 TNF-α	18	18	L, PL, S	Y	NR	Y	N	N	N	NR	Skin, Serum	4
LL37 IL-17 TNF-α IL-23 IL-1b IL-10 IL-32	24	9	L	Y	NR	NR	NR	N	Y (untreated)	NR	Skin	7
IL-6 IL-23 TNF-α R1 IL-1β IL-8 IL-10 IL-12p70 IL17A TNFR2 CRP ESR	74	22	Serum only	N	NR	Y	NR	N	N	NR	Serum	8
IFNg, IL-12p70,IL-1β IL-1α IL-17A IL-6 TNF-α TNF-β IL-16 IL-12/23p40 IL-10 IL-4 IL-13 IL-2 IL-15 IL-7 IL-5 GM-CSF VEGF	8	8	Wound Exudate	Y	NR	N	N	N	N	NR	Wound Exudate	6
IL-1B IL-6 IL-8 IL-10 IL-17A IL-23 TNFR1 TNFR2	19	19	Serum only	N	Y (Fasting)	N	N	N	Y (Adalimumab)	NR	Serum only	25
TNF-α, IL-1B, IL-6 IL-10 IL-17 IL-22 IL-1RA	120	24	Serum and Pus	Y	N	Y	N	N	Y (Etanercept)	NR	Serum Pus	5
IL-17 IL-1B IL-10 TNF-α	44	5	L, PL, U	N	N	N	N	N	N	NR	Skin	31
IL-17 Caspase1 NLRP3 S100A8 S100A9	22	Yes (NR)	L, PL, U, C	NR	NR	N	N	N	N	NR	Skin	30
TNF-α IL-1β IL-6 IFNg IL-17A IL-22	3	(Unknown)	S	Y	NR	N	N	N	N	NR	Serum	9
IL1-2p70 IL-23p19 IL-17	10	8	L, C	N	NR	N	N	N	Y (ceased 3/25 prior)	NR	Skin	32
IL-32 IL-32α IL-32β IL-32d IL-32g IFNg IL-17 IL-13	20	10	L, C, S	N	NR	Y	N	N	Y (ceased 8/52 prior)	NR	Skin, Serum	29
IL-36α IL-36β IL-36g IL-36RA	25	7	L, C, S	N	NR	N	N	N	Y (ceased 3/25 prior)	NR	Skin Serum	28
TNF-α IFNg IL-1β IL-6 IL-10 IL-19, IL-17A IL-22 IL-36b IL-12/23p40 IL-22 E Selectin P Selectin CXCL6 CXCL11 CX3CL1 CCL2 CCL18 CXCL9 sVEGFR1 MMP2 Cystatin C LCN2	10	16	L	Y	NR	N	N	N	N	NR	Skin Serum	27
IL-1β IL-2 IL-4 IL-5 IL-6 IL-8 IL-10 IL-12p70 TNF-α IFNg	20	6	L, PL, C	N	NR	Y	N	N	N	NR	Skin	26
IL-1α, IL-8	10	10	S	N	NR	N	N	N	Y	NR	Serum	33

Table 2: Critical Evaluation of Methodology of Studies Included in This Review Key:L= Lesional, PL= Perilesional, U= Uninvolved, C= Control S=Serum, Y=Yes, N=No, NR= Not Reported,

Table 4. Reported cytokine results of studies included in this systematic review.

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
IL-1α			1126			2549		Le:Ce	P= 0.53			6
			0.2			0.1	NR	L:C	NS			26
	772.0	697.2						HSs:Cs	NS			33
IL-1RA			44.0			29.6	1.5	L:C	P= 0.0112			26
IL-1β	0.9	0.4						HSs:Cs	P=0.801			8
			862.5			1503		Le:Ce	P= 0.69			6
								L:C	NS	Lpa:C	NS	25
			SERUM ONLY					HSs:Cs	P= 0.044			5
			100	10	3	1	115 fold	L:C	P= 0.001	PL:C	0.05	31
								L:U	P= 0.01	U:C	NS	
							R=0.7#	L:C	NS			7
			1.6			0.0	54.4	L:C	P= 0.0028			26
IL-4			6.56			9.77		Le:Ce	P= 0.54			6
			0.0			0.1		L:C	NS			7
IL-5			0.2			0.2		L:C	NS			7
			30.15			9.314		Le:Ce	P= 0.17			6
IL-6								L:C* L:C** L:C***	NS NS NS			4
	6.2	0.6						HSs:Cs	P= 0.001			8
			2377			5451		Le:Ce	NS			6
								L:C	P= 0.05	Lpa:C	0.05	25
			SERUM ONLY					HSs: Cs+++	P= 0.002			5
			124.4		101.9			L:C	NS			7
sIL-6R			16.3			4.4	3.7	L:C	P= 0.0028			7

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
IL-8	NR	NR	i69.6 / s67.6			64.9		Li:C	P<0.01	Ls:C	P<0.001	24
								L:C* L:C** L:C***	NS NS NS			4
	27.9	36.3						HSs:Cs	NS			8
								L:C	P= 0.05	Lpa:C	NS	25
			1401			12.0		L:C	NS			7
	1000	3000						L:C	P= 0.049			33
IL-10								L:C	P<0.05			4
	3.4	3.3						HSs:Cs	NS			8
			19.85			34.74		Le:Ce	NS			6
								L:C	P= 0.05	Lpa:C	0.05	25
			SERUM ONLY					HSs:Cs+	P= 0.0001			5
			SERUM ONLY					HSs:Cs++	P= 0.0001			5
			3.8	1.1		0.4	3-4	L:C	P= 0.01	PL:C	NS	31
								L:U	P= 0.01	U:C	NR	
	3	2						HSs:Cs	NS			27
			19.2			1.3	14.8	L:C	P= 0.0028			7
IL-11			78.6			7.2	11.0	L:C	P= 0.0056			7
IL-12p40			488.3			97.86		Le:Ce	P= 0.07			6
	75	75						HSs:Cs	NS			27
			0.5			0.4		L:C	NS			7
IL-12p70	3.4	0.6						HSs:Cs	P= 0.427			8
			9.412			15.02		Le:Ce	P= 0.609			6
			0.0			0.0		L:C	NS			7
IL-13			70.98			55.61		Le:Ce	P= 0.56			6
			0.0			0.1		L:C	NS			7

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
IL-15			24.5			5.61		Le:Ce	P= 0.18			6
			1.9			2.9		L:C	NS			7
IL-16			15277			15586		Le:Ce	P= 0.97			6
			22.3			4.2	5.3	L:C	P= 0.0028			7
IL-17								S:C	P<0.005			4
				SERUM ONLY		SERUM ONLY	SERUM ONLY		HSs:Cs+	0.014		5
				SERUM ONLY		SERUM ONLY	SERUM ONLY		HSs:Cs++	0.005		5
			150	45	1	1	149 fold	L:C	P= 0.05	PL:C	0.05	31
								L:PL	NS	U:C	0.05	
					No Quantification			L:C	↑(NS)	L:PL	No Diff	30
							R=0.66#		NS			27
IL-17A								L:C	P<0.005			4
	5.6	0.3						HSs:Cs	NS			8
			1006			32.7		Le:Ce	NS			6
								L:C	P= 0.05	Lpa:C	NS	25
	4	5						HSs:Cs	NS			27
			8.1	NR		1.1	7.3	L:C	P= 0.0056			26
IL-22								L:C	NS			4
	8.8	0.0						HSs:Cs	NS			8
IL-23								L:C	NS	Lpa:C	0.05	25
							R=0.68#		NS			7
IL-32	50ng/mL	1ng/mL	Only Normalised Values Provided				4 (skin) 50 (serum)	L:C	P= 0.01	HSs: Cs	p<0.05	29
IL-32α							3 fold	L:C	P= 0.01			29
IL-32β							2 fold	L:C	P= 0.05			29
IL-32g							Not elevated	L:C	P= 0.001			29
IL-32d							3 fold	L:C	NS			29

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
IL-36α			0.4	0.02		0.02		L:C	P=0.0174	PL:C	NS	3
	250	0				1	45.07 fold	L:C	P= 0.01			28
IL-36b			4.33	3.00		0.51		L:C	P= 0.0001	PL:C	0.0035	3
	15	4				1	1.45 fold	L:C	P= 0.25			28
IL-36g			3.64	0.83		0.49		L:C	P= 0.0161	PL:L	0.0302	3
	100	20				1	1.96 fold	L:C	P= 0.07			28
IL-36RA			0.46	0.28		0.06		L:C	P= 0.0001	PL:C	0.0003	3
	50	100	No Quantificaiton				No Increase	L:C	P= 0.10			28
IL-37			3.24	14.7		1.81		PL:L	P= 0.0002	PL:C	0.0001	3
IL-38			0.09	0.19		0.06		L:C	P= 0.0230	PL:C	0.0069	3
TNF-α			i69.4 66.6 [s]	NR		65.8	NR	Li:C	NS	Ls:C	NS	24
								L:C* L:C** L:C***	NS NS NS			4
				83.26			65.74	Le:Ce	P= 0.7			6
			SERUM ONLY	SERUM ONLY				HSs:Cs+	P=0.021			5
			2.2	1.3	0.6	0.7		L:C	P=0.01	PL:C	0.01	31
								L:PL	NS	U:C	NS	
			0.3			0.2	1.6	L:C	P=0.0336			26
TNF-β			9.24			1.65		Le:Ce	P=0.03			6
			0.4			0.4	NR	L:C	NS			26
sTNFR1	879.8	325.9						HSs:Cs	P <0.001			8
								L:C	NS	Lpa:C	0.05	25
			78.0			40.2	1.9	L:C	P= 0.0112			26

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
sTNFR2	927.9	527.4						HSs:Cs	P= 0.053			8
								L:C	P= 0.05	Lpa:C	0.05	25
			47.0			8.1	5.8	L:C	P= 0.0028			26
hBD1			0.019 0.021 0.018			0.058 0.077 0.095	0.3 0.3 0.2	L:C* L:C** L:C***	P= 0.240 P= 0.132 P= 0.026			4
hBD2			0.013 0.019 0.058			0.011 0.018 0.067	1.1 1.1 0.9	L:C* >L:C** L:C***	P= 0.937 P= 0.699 P= 0.937			4
hBD3			76.9[i] 75.7[s]			72.5	NR	Li:C	P<0.05	Ls:C	NS	24
			0.33 0.33 0.379			0.117 0.125 0.203	2.8 2.6 1.9	L:C* L:C** L:C***	P= 0.485 P= 0.394 P= 0.485			4
S100A7			i84.8 77.8 [s]			71.5	NR	Li:C	P<0.001	Ls:C	P<0.05	24
			1.516 1.625 2.297			0.177 0.354 0.707	8.6 4.6 3.2	L:C* L:C** L:C***	P= 0.009 P= 0.180 P= 0.132			4
S100A8			24.251 25.992 24.251			4.925 11.314 10.556	4.9 2.3 2.3	L:C* L:C** L:C***	P= 0.240 P= 0.537 P= 0.393			4
							NR	L:C	↑ (NS)	L:PL	(↑(NS)	30
S100A9			0.003 0.005 0.003			0.002 0.004 0.006	1.7 1.1 0.6	L:C* L:C** L:C***	NS NS NS			4
								L:C	↑ (NS)	L:PL	↑ (NS)	30
LL37			84.1[i] /80.9[s]			75.8		Li:C	P<0.05	Ls:C	NS	24
Lyzozyme			55.2[i] / 52.7[s]			59.6		Li:C	NS	Ls:C	P<0.05	24
MIF			77.8[i]/ 77.8[s]			70.7		Li:C	NS	Ls:C	P<0.01	24
αMSH			NR	i74.6[i] / 73.1[s]		NR	70.9	Li:C	P<0.01	Ls:C	P<0.01	24
MHC1			75.5[i]/74.7[s]			74.4		Li:C	NS	Ls:C	NS	24
RNase7			0.435 0.330 0.574			0.063 0.077 0.109	7.0 4.3 5.3	L:C* L:C** L:C***	P= 0.145 P= 0.589 P= 0.179			4
IP10			89.9			12.6		L:C* L:C** L:C***	P<0.05 P<0.005 P<0.05			4

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
CCL3			0.4			0.2	2.0	L:C	P= 0.0196			26
CCL5			- 46.1 -			- 6.2 -		L:C* L:C** L:C***	P<0.05 P<0.05 NS			4
			7.6			1.4	5.4	L:C	P= 0.0112			26
CCL20								L:C	P<0.005			4
CCL27								L:C	P<0.05			4
CRP	13.4	1.2						HSs:Cs	p<0.001			8
								L:C	P= 0.05	Lpa:C	0.05	25
ESR	29.5	10.2						HSs:Cs	<0.001			8
								L:C	P= 0.05	Lpa:C	0.05	25
IFNg							R=0.7	L:C	NS			7
							<5% Normal	HSs:Cs	↑ (NS)			9
			1418			102.5		Le:Ce	P= 0.027			6
								HSs:Cs	P<0.05	L:C	P<0.05	4
GMCSF			78.45			82.13		Le:Ce	P= 0.96			6
			0.4			0.0	NR	L:C	NS			26
VEGF			632.1			1544		Le:Ce	P= 0.23			6
sVEGFR1	60	60						HSs:Cs	NS			27
Caspase 1						No Quanti	No Quanti	L:C	↑ (NS)	L:PL	↑ (NS)	30
NLRP3						No Quanti	No Quanti	L:C	↑ (NS)	L:PL	NS	30
CAMP							4	L:C	NS			7
Uteroglobulin	20	20						HSs:Cs	NS			27
Cystatin C	0.85	0.8						HSs:Cs				27
LCN2	90	40	0.5		0.02			HSs:Cs	<0.001	L:C	<0.001	27
BD2	0.9	1						HSs:Cs	NS			27
MMP2	200	210						HSs:Cs	<0.05			27
BLC			8.1			0.58	10.5	L:C	P= 0.0056			26

Target Cytokine	Mean Level in Patient Serum (pg/mL)	Mean Level in Control Serum (pg/mL)	Mean Level in Lesional Tissue (pg/mL)	Mean Level in Perilesional Tissue (pg/mL)	Mean Uninvolved Tissue Levels (pg/mL)	Mean Control Tissue Levels (pg/mL)	Fold Increase	Comparison and Significance		Comparison and Significance		Study Reference
ICAM-1			98.7			31.9	3.1	L:C	P= 0.0028			26
Eotaxin			0.1			0.1	NR	L:C	NS			26
Eotaxin2			3.9			2.5	NR	L:C	NS			26
CXCL6	160	140							NS			27
CXCL9			219.8			13.8	16	L:C	P= 0.0028			26
CXCL11	0.4	0.4							NS			27
CX3CL1	0.9	1							NS			27
I-309			0.4			0.3	NR	L:C	NS			26
MCP1			47.5			37.1	NR	L:C	NS			26
M-CSF			0.4			0.2	NR	L:C	NS			26
MIP1b			16.1			5.8	NR	L:C	NS			26
MIP1d			0.1			0.1	NR	L:C	NS			26
PDGF			0.5			0.2	NR	L:C	NS			26
TIMP1			260.1			166.2	NR	L:C	NS			26
TIMP2			989.2			997.3	NR	L:C	NS			26

Key: L= Lesional ; PL= Perilesional; C= Control; NS= Not Significant ; HSs= HS Serum; Cs= Control Serum; HSe= HS Exudate; Ce= Control Exudate; I = Inflamed lesional skin, S= Scarred lesional skin, #= Vs CAMP, *= NT (Non-Treated) Samples ,** = Stimulation by Pam2CSK4 Lipopeptide,*** Stimulation by Muramyl Dipeptide (MDP), + Heat Killed Candida Albicans; ++ Heat Killed Staph Aureus, +++ Lipopolysaccharide;

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T-cell axis. There is an uncertain role for other T-cell subsets in HS. Increased production of CXCL9 and IP-10 (CXCL10) are often linked to production of IFN-γ from T_h1 T-cells in inflammatory sites, but IL-26 or IL-29, which are also cytokines produced by T_h17 T-cells are alternative activators of STAT1 and CXCL9 production. IL-32 production in HS may also be linked to a T-cell subset that produces this cytokine. Low production of T_h2 associated cytokines (IL-4, IL-5, or IL-13) has been measured in HS, suggesting an unlikely role of this T-cell subset. Likewise, the presence and function of T regulatory cells (Tregs) in HS lesions needs further study. IL-10 which is elevated in HS could be produced by either Tregs or the cDC1 (BDCA3+) DC subset, but levels may be inadequate to control tissue inflammation. At present, dendritic cell subsets are also incompletely characterized in HS. Potential sources of IL-12 or IL-23 are CD11c+ DCs, which includes the tissue resident BDCA-1+ (cDC2) subset and less mature inflammatory DCs, which are abundant cells in inflammatory lesions of psoriasis or atopic dermatitis but have not been investigated in HS. Cytokine contributions by other cell types such as innate lymphoid cells, macrophages, mast cells, and other leukocytes also remains to be determined.

Cytokine analysis methods

The methodologies of cytokine analysis varied widely (Table 5). 92 results were produced using electrochemical luminescence (ECL) procedures from three separate systems and manufacturers. 62 results were produced using ELISA. 18 results[4] were performed with either ELISA or ECL but not further specified. 15 results were produced using polymerase chain reaction (PCR) with three separate systems from three manufacturers. Four discrete cytokines (IL-10, IL-17, TNF-α and IFN-γ) were analysed using all three techniques (ECL, ELISA and PCR), whilst 15 discrete cytokines (IL-6, IL-8, IL12p40, IL-17A, IL-22, IL-23, S100A7, S100A8, S100A9, RNAse7, IP-10, CCL5, CCL20, CCL27) were analysed using ELISA and ECL only. We note IL-17 levels may well be below the lower limit of quantification with ELC and ELISA based approaches, with only the Singulex platform having the ability to quantify levels of IL-17 present in blood and serum of normal subjects.

Assessment of bias

Assessment of bias is presented in Table 6. Two of the 14 questions regarding participation rate and loss to follow up were considered not applicable. All included studies identified clear objectives and a clearly defined study population. No clear inclusion or exclusion criteria were specified for 17 of the 19 studies. Power estimation was made for one study[33], and recording of all exposures (disease activity, comorbidities etc) were made prior to assessment of the outcomes (cytokine levels). The timeframe of analysis was sufficient to identify an association, but only 10 of the 19 studies (52.6%) documented different levels of exposures (disease severity, metabolic comorbidities, family history etc). There were no serial measures of cytokine levels in the majority of studies. Only three studies[5,25,33], examining cytokine levels after monoclonal antibody administration has measurements at two distinct time points. Outcomes of interest (cytokine levels) were measured consistently within studies, however there was great variance in the methods of measurement and analysis between studies (Table 5). No studies took into account known confounding variables into analysis of their results by stratification or regression analyses.

Assessment of heterogeneity

36 of the 81 identified cytokines or inflammatory proteins were assessed by more than 1 study. 23 of those cytokines had raw data available. No studies had sufficient measures of spread in order to calculate I^2 measure of heterogeneity and so chi-squared statistic was used as an alternate marker of heterogeneity (Table 7) along with a funnel plot (Figure 3). In total, 18 individual cytokines (78.2%) were found to demonstrate heterogeneity. Only eight cytokines (Serum IL-10, Lesional IL-1α, IL-12p70, hBD1, hBD2, hBD3, S100A9 and GMCSF) illustrated homogeneity. Due to this high level of heterogeneity and concerns regarding the methodological quality of included studies, meta-analysis was not deemed appropriate to perform.

Discussion

The overall quality of reporting in the identified studies was low with little consistency between methodologies and cytokines examined. There was also great variability in the ages, genders, comorbidities, associated conditions and treatments of the patients included in these studies. This was again reflected in the high number of cytokines with statistical heterogeneity (Table 7). The studies presenting conflicting data are often those studies with lower numbers of patients as well as lack of matched controls and/or lack of stratification by treatment. Meta-analysis using individual patient data would be required in order to account for these factors and re-assess the relationship between lesional and control cytokine levels.

In assessing the relationship between lesional and peri-lesional tissue, it has been demonstrated by many authors that different cytokines are present in peri-lesional tissue as opposed to lesional tissue. The definition of peri-lesional tissue is fairly consistent in the studies examined being 2cm from an active HS nodule on unaffected skin. However, no studies reported ultrasound examination of the peri-lesional skin to ensure that subclinical extension of the adjacent nodule (either in the dermis or the subcutaneous tissue) was being inadvertently sampled. This is an important differentiation to make in terms of identifying the subclinical pathogenic processes that precipitate this disease.

The raw data collated illustrates a number of paradoxically elevated levels of control cytokines (IL-15, IL-16) (Table 4). Many of these control readings lie near the lower detection limit of specific assays in individual papers, and thus the possibility of erroneously elevated control readings cannot be excluded. The wide interquartile ranges of studies which did report individual patient data[7], suggest that analyzing aggregate data is not optimal and is prone to misrepresentation of the relationship between clinical disease, comorbidities and cytokine levels. Furthermore, high levels of heterogeneity within the measurements of individual cytokines suggest that examination of and correction for other variables or confounders is required.

Table 5. Cytokine analysis methodology of studies included in this review.

Cytokine	Method	Details	Study
IL-1α	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
IL-1ra	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
IL-1β	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	5
	PCR	IL10, IL17A, IL1B, IL18 and NLRP3 was performed with predesigned Taqman gene expression assays (Applied Biosystems) on a Roche Light Cycler (Roche, Pleasanton, CA, U.S.A.)	31
	PCR	(Hs01555410_m1), ABI-Prism 7300 Sequence Detector System (Applied Biosystems	7
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
IL-4	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-5	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
IL-6	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA). The Milliplex MAP multiplex assay	25
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	5
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
sIL-6R	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-8	ELISA	pABG AHC0881 1:50 rabbit antihuman	24
	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	33
IL-10	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6

Cytokine	Method	Details	Study
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	5
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	5
	PCR	IL10, IL17A, IL1B, IL18 and NLRP3 was performed with predesigned Taqman gene expression assays (Applied Biosystems) on a Roche Light Cycler (Roche, Pleasanton, CA, U.S.A.)	31
	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-11	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-12p40	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-12p70	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-13	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-15	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-16	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	7
IL-17	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	5
	PCR	IL10, IL17A, IL1B, IL18 and NLRP3 was performed with predesigned Taqman gene expression assays (Applied Biosystems) on a Roche Light Cycler (Roche, Pleasanton, CA, U.S.A.)	31
	PCR	IL-17 (clone AF-317-NA; R&D Systems, Wiesbaden, Germany),	30
	PCR	IL-17 (Hs00174383_m1), ABI-Prism 7300 Sequence Detector System	27
IL-17A	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA). eBioscience, Paris, France	4
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26

Cytokine	Method	Details	Study
IL-22	ELISA	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA). eBioscience, Paris, France	4
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
IL-23	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	PCR	(Hs00992441_m1) ABI-Prism 7300 Sequence Detector System (Applied Biosystems	7
IL-32	PCR	IL-32 (Hs00992441_m1), ABI-Prism 7300 Sequence Detector System	29
IL-32α	PCR	IL-32a (Hs04353657_gH), ABI-Prism 7300 Sequence Detector System	29
IL-32β	PCR	IL-32b (Hs04353658_gH), ABI-Prism 7300 Sequence Detector System	29
IL-32g	PCR	IL-32c (Hs04353656_g1), ABI-Prism 7300 Sequence Detector System	29
IL-32d	PCR	IL-32d (Hs04353659_gH), ABI-Prism 7300 Sequence Detector System	29
IL-36α	ELISA	Rabbit polyclonal anti-IL-36a (C-terminal; ab180909), from Abcam, Cambridge, U.K. at 1 : 500 dilution.	3
	ELISA	IL-36a AF1078, RnD	28
IL-36β	ELISA	Rabbit polyclonal anti- IL-36b (C-terminal; ab180890) from Abcam, Cambridge, U.K. at 1 : 500 dilution.	3
	ELISA	AF1099, RnD	28
IL-36g	ELISA	Mouse monoclonal anti-IL-36c ab156783; (Abcam, Cambridge, U.K.) at 1 : 500 dilution.	3
	ELISA	AF2320, RnD	28
IL-36RA	ELISA	Rabbit polyclonal from Abcam, Cambridge, U.K. at 1 : 500 dilution.	3
	ELISA	AF1275, RnD	28
IL-37	ELISA	Rabbit polyclonal Abcam, Cambridge, U.K. at 1 : 500 dilution.	3
IL-38	ELISA	Rabbit polyclonal Abcam, Cambridge, U.K. at 1 : 500 dilution.	3
TNF-α	ELISA	TNF-alpha: 559071 mABG 1:10 mouse antihuman	24
	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ELISA	Cytokines were measured in duplicate by ELISA (R&D Minneap- olis, USA).	5
	PCR	Taqman gene expression assays (Applied Biosystems) on a Roche Light Cycler	31
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
TNF-β	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
sTNFR1	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
sTNFR2	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	8
	ECL	xMAP technology (Luminex Corporation, Austin, TX, USA)	25
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
hBD1	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4

Cytokine	Method	Details	Study
hBD2	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
hBD3	ELISA	ELISA 1 : 400; rabbit antihuman	24
	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
S100A7	ELISA	Psoriasin HL15-4 mAbG 1:20,000 mouse antihuman	24
	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
S100A8	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ELISA	S100A8 and S100A9 (monospecific affinity-purified rabbit antisera to S100A8 and to S100A9	30
S100A9	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ELISA	S100A8 and S100A9 (monospecific affinity-purified rabbit antisera to S100A8 and to S100A9	30
LL37	ELISA	Cathelicidin ab64892 pAbG 1:1000 rabbit antihuman	24
Lyzozyme	ELISA	Lysozyme A0099 pAbG 1:100 rabbit antihuman	24
MIF	ELISA	MIF MAB289 mABG 1:100 mouse antihuman	24
αMSH	ELISA	alpha MSH M09393 mABG 1:500 rabbit antihuman	24
MHC1	ELISA	MHC1 W6/32 mABG 1:50 mouse antihuman	24
RNase7	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
IP10	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
CCL3	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
CCL5	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
CCL20	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
CCL27	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
CRP	ECL	xMAP luminex Luminex Corporation, Austin, TX, USA	8
	ECL	xMAP luminex Luminex Corporation, Austin, TX, USA	25
ESR	ECL	xMAP luminex Luminex Corporation, Austin, TX, USA	8
	ECL	xMAP luminex Luminex Corporation, Austin, TX, USA	25
IFNg	PCR	(Hs00174143_m1), ABI-Prism 7300 Sequence Detector System (Applied Biosystems)	7
	ELISA	ELISA kits from Sanquin (Amsterdam, The Nether- lands)	9
	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6

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Cytokine	Method	Details	Study
	ELISA/ ECL	ELISA (Quantikine; R&D Systems) or Luminex assay (Millipore, Billerica, MA).	4
GMCSF	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
	ELISA	Quantibody Human Inflammation array 3 (RayBiotech Inc., Norcross, GA, U.S.A.).	26
VEGF	ECL	Meso Scale Discovery electrochemiluminescent assay (MSD, Meso Scale Diagnostics, Rockville, MD MSD V-Plex cytokine panel 1 and the V-plex proinflammatory panel 1	6
sVEGFR1	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
Caspase 1	ELISA	Kelly *et al.* Caspase-1 fluorochrome inhibitor of caspases (FLICA) (ImmunoChemistry Technologies, Bloomington, MN, U.S.A.	30
NLRP3	PCR	Kelly IL10, IL17A, IL1**B**, IL18 and NLRP3 was performed with predesigned Taqman gene expression assays (Applied Biosystems) on a Roche Light Cycler (Pleasanton, CA, U.S.A.)	30
CAMP	PCR	(Hs00189038_m1) ABI-Prism 7300 Sequence Detector System (Applied Biosystems)	7
Uteroglob	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
Cystatin C	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
LCN2	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
BD2	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
MMP2	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
BLC	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
ICAM-1	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
Eotaxin	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
Eotaxin2	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
CXCL6	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
CXCL9	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
CXCL11	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
CX3CL1	ELISA	Quantikine enzyme-linked immunosorbent assay (ELISA) systems from Bio-Techne	27
I-309	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
MCP1	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
M-CSF	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
MIP1b	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
MIP1d	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
PDGF-BB	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
TIMP1	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26
TIMP2	ECL	(CBA Human Inflammation kit and CBA Human TH1/TH2 Cytokine kit; BD Biosciences, Franklin Lakes, NJ, U.S.A.) Analyssi: FACSCalibur (BD Biosciences)	26

Table 4: Antibodies Used for Identification of Cytokines in Studies Included in this Systematic Review. ECL: Electrochemicoluminescence

Table 6. Risk of bias across studies included in this review.

Study Reference	1. Was the research question or objective in this paper clearly stated?	2. Was the study population clearly specified and defined?	3. Was the participation rate of eligible persons at least 50%?	. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	5. Was a sample size justification, power description, or variance and effect estimates provided?	6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	10. Was the exposure(s) assessed more than once over time?	11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	12 Were the outcome assessors blinded to the exposure status of participants?	13. Was loss to follow-up after baseline 20% or less?	14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
Moran *et al.*[2]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Emelianov *et al.*[24]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Hessam *et al.*[3]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Hotz *et al.*[4]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Thomi *et al.*[7]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Jimenez-Gallo *et al.*[8]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Banerjee *et al.*[6]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Jimenez-Gallo *et al.*[25]	Y	Y	N/A	Y	N	Y	Y	N	Y	N	Y	NR	N/A	N

Study Reference	1. Was the research question or objective in this paper clearly stated?	2. Was the study population clearly specified and defined?	3. Was the participation rate of eligible persons at least 50%?	. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	5. Was a sample size justification, power description, or variance and effect estimates provided?	6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	10. Was the exposure(s) assessed more than once over time?	11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	12 Were the outcome assessors blinded to the exposure status of participants?	13. Was loss to follow-up after baseline 20% or less?	14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
Kanni *et al.*[5]	Y	Y	N/A	N	N	Y	Y	Y	Y	Y	Y	NR	N/A	N
Kelly *et al.*[31]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Lima *et al.*[30]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Ten Oever *et al.*[9]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Schlapbach *et al.*[32]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Thomi *et al.*[29]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Thomi *et al.*[28]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Wolk *et al.*[27]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Van der Zee *et al.*[26]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Kanni *et al.*[33]	Y	Y	N/A	Y	Y	Y	Y	Y	Y	N	Y	NR	N/A	N

Key: Y = Yes; N= No, NR= Not Reported N/A = Not Applicable

Table 7. Table of heterogeneity of cytokine studies by chi-squared tests for homogeneity.

Cytokine	Chi Squared	P
IL1a Lesional	**0.3525**	**p=0.552705**
IL1b Lesional	153.5947	p<0.00001
IL4 Lesional	4.3992	P=0.035955
IL5 Lesional	15.1692	P=0.000098
IL6 Lesional	461.9724	P<0.00001
IL8 Lesion	846.6251	P<0.0001
IL8 Serum	94.4212	P<0.0001
IL10 Lesion	90.3211	P<0.0001
IL10 Serum	**0.1595**	**P=0.689624**
IL12p40 Lesional	4.9618	P=0.025913
IL12p70 Lesional	**2.2116**	**P=0.136973**
IL13 Lesional	5.4163	P=0.019949
IL15 Lesional	39.2837	P<0.00001
IL16 Lesional	126.1959	P<0.00001
IL17A Lesional	22.6668	P<0.00001
IL17A Serum	19.1621	P=0.000012
TNFa Lesional	6.9761	P=0.030561
TNFb Lesional	7.4004	P=0.006521
hBD1 Lesional	**2.3317**	**P=0.311656**
hBD2 Lesional	**0.6488**	**P=0.722954**
hBD3 Lesional	**1.0314**	**P=0.597084**
S100A7 Lesional	621.2537	P<0.00001
S100A8 Lesional	19.6371	P=0.000054
S100A9 Lesional	**1.27**	**P=0.529927**
RNAse 7	6.7263	P=0.034626
GMCSF Lesional	**1.9405**	**P=0.163611**

Methodological quality

Regarding methods of cytokine analysis, a number of authors have identified variability in cytokine levels measured with different forms of multiplex assays as well as traditional ELISA methods[35–39]. Different methods of cytokine analysis are known to be prone to variability, with some cytokines more sensitive than others. For example, IFN-γ and IL-1β were overestimated compared with ELISA methods[37], whilst IL-6 levels were underestimated[37]. IL-6 levels when compared across four different multiplex assays showed significant variation in detectable range, accuracy and responsiveness[36]. The correlation of TNF-α between ELISA and Multiplex assays was also poor (r=0.31)[36]. Issues also exist with minimum detectable levels of cytokines with specific bead-based arrays[36] As an example, minimal detectable dose readings reported for IL-12p70 using some multiplex arrays[39] are higher than the levels reported in lesional HS samples[6]. Therefore, whilst the general trends in the level of consistently elevated or suppressed cytokines in HS are reliable, the quantification of individual cytokines as well as the relationship between comorbidities and cytokine levels requires further research with consistent, reliable and accurate methodologies in order to further dissect the inflammatory cascade in this disease.

Keratinocyte mediated inflammatory pathways

The majority of elevated cytokines and inflammatory proteins identified in lesional skin of HS (TNF-α, IL-1β, IL-6, IL-8, IL-11, IL-23, IL-17A, IL-33, IL-36, LL-37, S100A7, S100A8, S100A9, GM-CSF, TGF-β, hBD2, hBD3, CCL3, CXCL9, CXCL11, PDGF, CCL5, CCL-20, MIF, GM-CSF and LCN2) are those known to be produced by keratinocytes, as well as perpetuating a self-amplification pathway[34] (Figure 2). Additionally T-cells produce IL-17A, IL-17F, IL-26, IL-29, and IFN-γ; dendritic cells produce IL-12, IL-23 and possibly IL-39; neutrophils produce S100A8

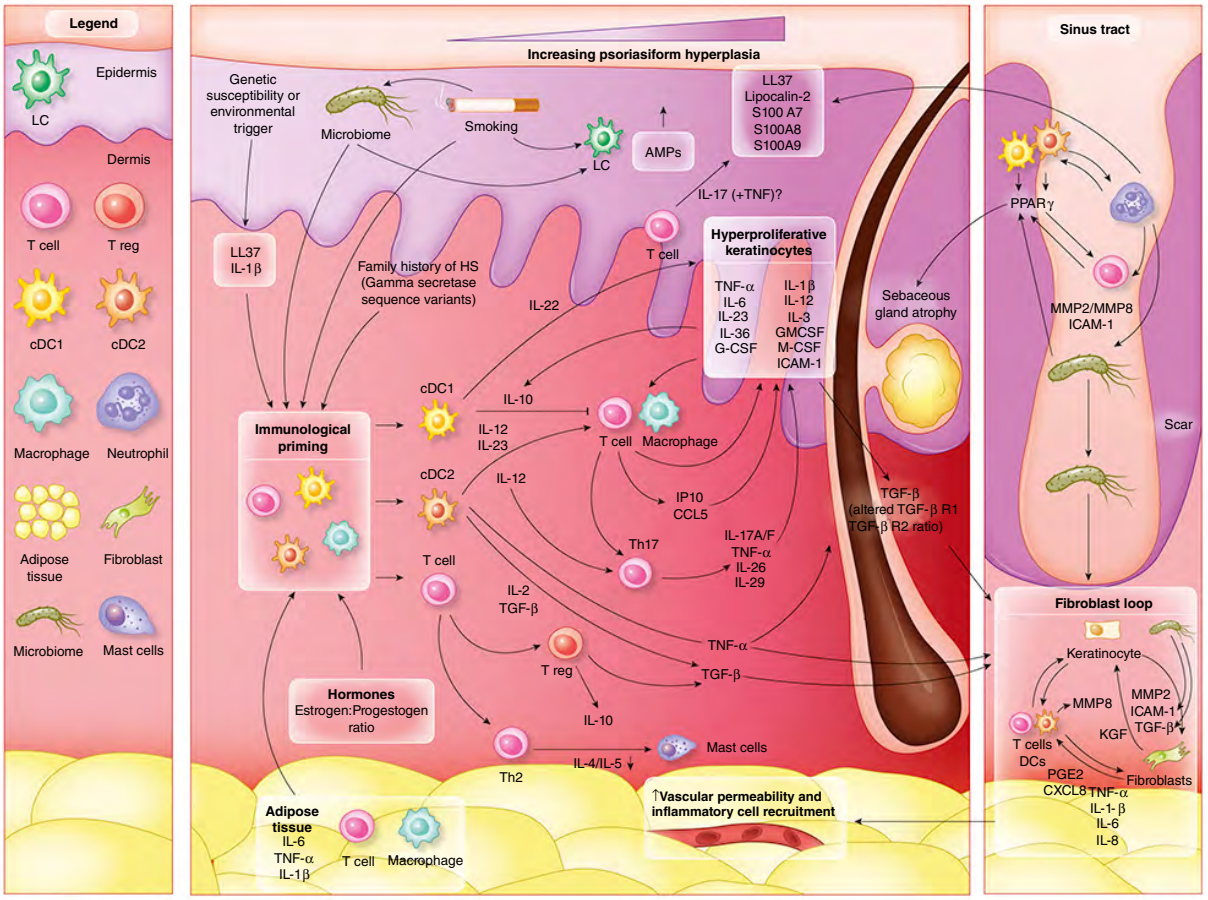

Figure 2. Inflammatory pathways in hidradenitis suppurativa, a schematic representation of the results identified in this systematic review. Immunological 'priming' occurs due to the contribution of adipose tissue, genetic susceptibility, smoking-related inflammatory mediators and obesity related pro-inflammatory signals and the composition of the microbiome. Increased activity of cDC1, cDC2 and T cells lead to both keratinocyte hyperplasia via the actions of IL-12 and IL-23, as well as a Th17 predominant immune response. Alterations of antimicrobial peptides (AMP's) also occur throughout the epidermis. The dermal inflammation interacting with the hyperplastic epidermis result leads to a self-perpetuating inflammatory feed forward mechanism mediated by IL-36, Il-1B and TNF-a. The development of scarring and sinus tracts is associated with MMP2, ICAM-1 and TGF-Beta, with possible augmentation of ICAM-1 and TGF-B signaling via specific components of the microbiome. TNF-a, PGE2 and CXCL2 then lead to additional feed forward mechanisms perpetuating the inflammatory cycle.

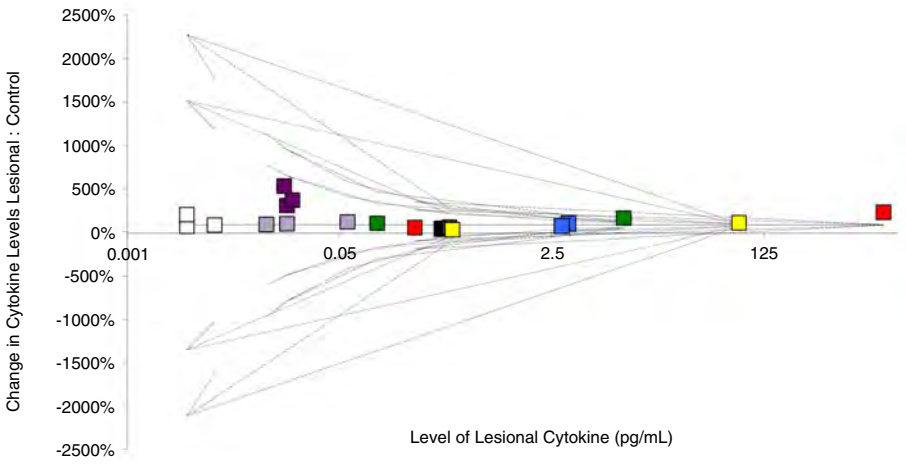

Figure 3. Funnel plot of selected cytokine in lesional and control samples of hidradenitis suppurativa. IL-1a = Red, IL-10 = Blue, IL-12p70 = Green, hBD1 = Purple, hBD2 = light purple, hBD3 = Black, S100A9 = White, GMCSF = Yellow.

and S100A9 (calgranulin); and innate lymphoid cells also contribute IFN-γ, IL-17A and IL-17F. This inflammatory model has been well documented and explored in both psoriasis and atopic dermatitis[34,40]. The psoriasiform epidermal hyperplasia seen in HS (mediated by IL-17 and maintained by IL-23-mediated T_h17 stimulation)[34] reflects this common inflammatory pathway.

The other elevated non-keratinocyte produced cytokines in HS (IL-4, IL-5, IL-10, IL-16, IL-17A, IL-22, IL-32, IL-36, hBD1), are produced by a combination of dendritic cells, monocytes, neutrophils and CD4+ T cells. IL-4 and IL-5 as key cytokines in the T_h2 axis are consistent with the findings of Mast cells in HS[41], as well as the pruritus, which is frequently reported by patients. IL-10 in HS is produced by Treg cells[2] (although dendritic cells may also be a source), and whilst quantitatively the IL-10 signal appears paradoxically elevated, it can be explained by the up-regulation of T cells including Treg cells, which although significantly elevated from baseline, are not elevated enough in comparison to T_H17/IL-17/IL-22 signal to counteract this strong pro-inflammatory cascade[2]. Further exploration of these cytokines may reveal the initial trigger(s) of the inflammatory cascade in HS, or correlations with known pro-inflammatory comorbidities.

Insights into pathogenesis of HS

In light of investigations in psoriasis and atopic dermatitis, the role of dendritic cells in HS needs to be clarified, as dendritic cell influx has been reported in histological studies[41,42], and they may contribute to the high IL-10 and IL-15 levels reported. IL-32 is a second cytokine produced by dendritic cells, but has only been reported in one study[29]. Further research into the functional role of IL-32 in the activity of dendritic cells in HS would be of value. The role of IL-20, IL-22, IL-24 and IL-26 needs further clarification. IL-19, TSLP and CCL17 (TARC) have not yet been examined in HS and this is required in order to further explore the role of dendritic cell, monocyte and T cell activation and migration in this disease.

It is well established that smoking, obesity and diabetes are strongly associated with HS[13–19,42,43]. The immunological effects of smoking include increase in number and responsiveness of dendritic cells, altered function of Treg cells and activation of Th17 pathways[44], whilst obesity and diabetes can result in production of IL-1β, IL-6 and TNF-α through activated macrophages in adipose tissue[45,46]. These potential mechanistic pathways (which may prime or contribute towards inflammation in HS) require validation in functional studies. However, if they are a significant contributor to inflammation, the presence or absence of these comorbidities need to be considered in future cytokine studies as confounding variables in order to identify significant biochemical markers independent of these other pro-inflammatory states that reflect the pathogenesis of HS.

The role of the microbiome[42,43] in stimulating chronic inflammation has parallels in diabetes[47] and colonic inflammation[48] and the presence of *Porphyromonas* and *Peptoniphilus* species has been associated with a subpopulation of patients with HS[42]. *Porphyromonas* has been associated with systemic inflammation and atherosclerosis through aberrant toll-like-receptor 4 signalling[48] and is not part of the natural cutaneous flora[43]. Altered cutaneous and gastrointestinal microbiome can also act via microbiome metabolites (including lipopolysaccharides, short chain fatty acids and bile salts)[49] through stimulation of myeloid dendritic cells via G Protein Coupled Receptors (including GPR41, GPR43 and GPR109A)[49,50]. The microbiome may be implicated as a trigger factor for the initial inflammatory cascade in HS in a proportion of patients. Similarly, the presence of genetic polymorphisms as reported in HS[51] have the potential to up-regulate inflammatory activity through shedding of IL-6R, IL-15R, TNF-α[52] as well as up-regulating the response of dendritic cells to LPS stimulation via ADAM17 (which has been demonstrated to be elevated in a published gene expression study of HS)[53]. These pathways may be involved prior to the activation of keratinocyte-mediated inflammation, and hence, may reveal novel targets for new interventions to control the disease prior to the onset of destructive inflammation.

Limitations, interpretation and generalisability

The limitations to this study include the high degree of methodological variability (Table 5) and high impact of bias (Table 6) within the included studies. The lack of individual patient data has also prevented any further analysis into the contribution of comorbidities such as smoking and obesity to variable levels of cytokines in lesional tissue and/or serum. This, along with the high level of heterogeneity in many cytokines (Table 7), has resulted in analyses of the collated data being limited to descriptive analyses only and limited the generalisability of results.

Conclusions

Through this review we have catalogued the various cytokines that have been reported as elevated in lesional, peri-lesional tissue, serum or exudate of HS patients. We have also identified those cytokines with inconsistent results and identified methodological factors that may explain variability in findings. We have identified a number of missing links in disease pathogenesis with respect to cytokine actions and pathways that must be addressed in future work. Areas for further investigation include the role of dendritic cells in HS, the contribution of obesity, smoking, diabetes and the microbiome to cytokine profiles in HS, and examining the natural history of the disease through longitudinal measurements of cytokines over time.

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

OSF: Extend data. Data Collection Sheet Cytokine. Review HS. https://doi.org/10.17605/OSF.IO/N2E7A[22]

Reporting guidelines

OSF: PRISMA checklist for 'A systematic review and critical evaluation of inflammatory cytokine associations in hidradenitis suppurativa'. https://doi.org/10.17605/OSF.IO/N2E7A[22]

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References

1. Hoffman LK, Ghias MH, Lowes MA: **Pathophysiology of hidradenitis suppurativa.** *Semin Cutan Med Surg.* 2017; **36**(2): 47–54.
PubMed Abstract | Publisher Full Text

2. Moran B, Sweeney CM, Hughes R, *et al.*: **Hidradenitis Suppurativa is characterized by Dysregulation of the Th17:Treg Cell Axis, which is Corrected by Anti-TNF Therapy.** *J Invest Dermatol.* 2017; **137**(11): 2389–2395.
PubMed Abstract | Publisher Full Text

3. Hessam S, Sand M, Gambichler T, *et al.*: **Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop.** *Br J Dermatol.* 2018; **178**(3): 761–767.
PubMed Abstract | Publisher Full Text

4. Hotz C, Boniotto M, Guguin A, *et al.*: **Intrinsic Defect in Keratinocyte Function Leads to Inflammation in Hidradenitis Suppurativa.** *J Invest Dermatol.* 2016; **136**(9): 1768–1780.
PubMed Abstract | Publisher Full Text

5. Kanni T, Tzanetakou V, Savva A, *et al.*: **Compartmentalized Cytokine Responses in Hidradenitis Suppurativa.** *PLoS One.* 2015; **10**(6): e0130522.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Banerjee A, McNish S, Shanmugam VK: **Interferon-gamma (IFN-γ) is Elevated in Wound Exudate from Hidradenitis Suppurativa.** *Immunol Invest.* 2017; **46**(2): 149–158.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Thomi R, Schlapbach C, Yawalkar N, *et al.*: **Elevated levels of the antimicrobial peptide LL-37 in hidradenitis suppurativa are associated with a Th1/Th17 immune response.** *Exp Dermatol.* 2018; **27**(2): 172–177.
PubMed Abstract | Publisher Full Text

8. Jiménez-Gallo D, de la Varga-Martínez R, Ossorio-García L, *et al.*: **The Clinical Significance of Increased Serum Proinflammatory Cytokines, C-Reactive Protein, and Erythrocyte Sedimentation Rate in Patients with Hidradenitis Suppurativa.** *Mediators Inflamm.* 2017; **2017**: 2450401.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Ten Oever J, van de Veerdonk FL, Joosten LA, *et al.*: **Cytokine Production Assays Reveal Discriminatory Immune Defects in Adults with Recurrent Infections and Noninfectious Inflammation.** *Clin Vacc Immunol.* 2014: **21**(8): 1061–1069.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Canoui-Poitrine F, Le Thuaut A, Revuz JE, *et al.*: **Identification of three hidradenitis suppurativa phenotypes: latent class analysis of a cross-sectional study.** *J Invest Dermatol.* 2013; **133**(6): 1506–11.
PubMed Abstract | Publisher Full Text

11. van der Zee HH, Jemec GB: **New Insights into the diagnosis of hidradenitis suppurativa: Clinical presentations and phenotypes.** *J Am Acad Dermatol.* 2015; **73**(5 Suppl 1): S23–26.
PubMed Abstract | Publisher Full Text

12. Zhou X, Fragala MS, McElhaney JE, *et al.*: **Conceptual and Methodological Issues relevant to Cytokine and inflammatory marker measurements in clinical research.** *Curr Opin Clin Nutr Metab Care.* 2010; **13**(5) : 541–547.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Ergun T: **Hidradenitis Suppurativa and the Metabolic Syndrome.** *Clin Dermatol.* 2018; **36**(1): 41–47.
PubMed Abstract | Publisher Full Text

14. Garg A, Birabaharan M, Strunk A: **Prevalence of type 2 diabetes mellitus among patients with hidradenitis suppurativa in the United States.** *J Am Acad Dermatol.* 2018; **79**(1): 71–76.
PubMed Abstract | Publisher Full Text

15. Lukach AJ, Saul MI, Ferris LK, *et al.*: **Risk Factors for Hidradenitis Suppurativa in Patients with Inflammatory Bowel Disease.** *Dig Dis Sci.* 2018; **63**(3): 755–760.
PubMed Abstract | Publisher Full Text

16. Garg A, Papagermanos V, MIdura M, *et al.*: **Incidence of Hidradenitis Suppurativa among tobacco smokers: a population-based retrospective analysis in the U.S.A.** *Br J Dermatol.* 2018; **178**(3): 709–714.
PubMed Abstract | Publisher Full Text

17. Sgambato JA, Jones BA, Caraway JW, *et al.*: **Inflammatory profile analysis reveals differences in cytokine expression between smokers, moist snuff users, and dual users compared to non-tobacco consumers.** *Cytoine.* 2018; **107**: 43–51.
PubMed Abstract | Publisher Full Text

18. Spoto B, Di Betta E, Mattace-Raso F, *et al.*: **Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity.** *Nutr Metab Cardiovasc Dis.* 2014; **24**(10): 1137–43.
PubMed Abstract | Publisher Full Text

19. Rehman K Akash MS: **Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked?** *J Biomed Sci.* 2016; **23**(1): 87.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Frew J, Hawkes J Krueger J: **Cytokine Studies in Hidradenitis Suppurativa: A Systematic Review of Observational Studies.** PROSPERO 2018: CRD42018104664, Accessed 30 July 2018.
Reference Source

21. Moher D, Liberati A, Tetzlaff J, *et al.*: **Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.** *PLoS Med.* 2009; **6**(7): e1000097.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Frew J: **A Systematic Review and Critical Evaluation of Inflammatory Cytokine Associations in Hidradenitis Suppurativa**. 2018.
http://www.doi.org/10.17605/OSF.IO/N2E7A

23. NIH NIoH: **Quality Assessment Tool for Observational Cohort and Cross Sectional Studies**. 2018; Accessed 30 July 2018.
Reference Source

24. Emelianov VU, Bechara FG, Gläser R, *et al.*: **Immunohistological pointers to a possible role for excessive cathelicidin (LL-37) expression by apocrine sweat glands in the pathogenesis of hidradenitis suppurativa/acne inversa.** *Br J Dermatol.* 2012; **166**(5): 1023–1034.
PubMed Abstract | Publisher Full Text

25. Jiménez-Gallo D, de la Varga-Martínez R, Ossorio-García L, *et al.*: **Effects of adalimumab on T-helper-17 lymphocyte- and neutrophil-related inflammatory serum markers in patients with moderate-to-severe hidradenitis suppurativa.** *Cytokine.* 2018; **103**: 20–24.
PubMed Abstract | Publisher Full Text

26. van der Zee HH, Laman JD, de Ruiter L, *et al.*: **Adalimumab (antitumour necrosis factor-α) treatment of hidradenitis suppurativa ameliorates skin inflammation: an *in situ* and *ex vivo* study.** *Br J Dermatol.* 2012; **166**(2): 298–305.
PubMed Abstract | Publisher Full Text

27. Wolk K, Wenzel J, Tsaousi A, *et al.*: **Lipocalin-2 is expressed by activated granulocytes and keratinocytes in affected skin and reflects disease activity in acne inversa/hidradenitis suppurativa.** *Br J Dermatol.* 2017; **177**(5): 1385–1393.
PubMed Abstract | Publisher Full Text

28. Thomi R, Kakeda M, Yawalkar N, *et al.*: **Increased expression of the interleukin-36 cytokines in lesions of hidradenitis suppurativa.** *J Eur Acad Dermatol Venereol.* 2017; **31**(12): 2091–2096.
PubMed Abstract | Publisher Full Text

29. Thomi R, Yerly D, Yawalkar N, *et al.*: **Interleukin-32 is highly expressed in lesions of hidradenitis suppurativa.** *Br J Dermatol.* 2017; **177**(5): 1358–1366.
PubMed Abstract | Publisher Full Text

30. Lima AL, Karl I, Giner T, *et al.*: **Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa.** *Br J Dermatol.* 2016; **174**(3): 514–521.
PubMed Abstract | Publisher Full Text

31. Kelly G, Hughes R, McGarry T, *et al.*: **Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa.** *Br J Dermatol.* 2015; **173**(6): 1431–1439.
PubMed Abstract | Publisher Full Text

32. Schlapbach C, Hänn T, Yawalkar N, *et al.*: **Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa.** *J Am Acad Dermatol.* 2011; **65**(4): 790–8.
PubMed Abstract | Publisher Full Text

33. Kanni T, Argyropoulou M, Spyridopoulos T, *et al.*: **MABp1 Targeting IL-1α for**

Moderate to Severe Hidradenitis Suppurativa Not Eligible for Adalimumab: A Randomized Study. *J Invest Dermatol.* 2018; **138**(4): 795–801.
PubMed Abstract | Publisher Full Text

34. Hawkes JE, Chan TC, Krueger JG: **Psoriasis pathogenesis and the development of novel targeted immune therapies.** *J Allergy Clin Immunol.* 2017; **140**(3): 645–653.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Bastarache JA, Koyama T, Wickersham NE, *et al.*: **Validation of a multiplex electrochemiluminescent immunoassay platform in human and mouse samples.** *J Immunol Methods.* 2014; **408**: 13–23.
PubMed Abstract | Publisher Full Text | Free Full Text

36. Thompson DK, Huffman KM, Kraus WE, *et al.*: **Critical appraisal of four IL-6 immunoassays.** *PLoS One.* 2012; **7**(2): e30659.
PubMed Abstract | Publisher Full Text | Free Full Text

37. de Koning L, Liptak C, Shkreta A, *et al.*: **A multiplex immunoassay gives different results than singleplex immunoassays which may bias epidemiologic associations.** *Clin Biochem.* 2012; **45**(10–11): 848–851.
PubMed Abstract | Publisher Full Text

38. Tighe PJ, Ryder RR, Todd I, *et al.*: **ELISA in the multiplex Era: Potentials and Pitfalls.** *Proteomics Clin Appl.* 2015; **9**(3–4): 406–22.
PubMed Abstract | Publisher Full Text

39. dupont NC, Wang K, Wadhwa PD, *et al.*: **Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants.** *J Reprod Immunol.* 2005; **66**(2): 175–191.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Brunner PM, Guttman-Yassky E, Leung DY: **The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies.** *J Allergy Clin Immunol.* 2017; **139**(4S): S65–S76.
PubMed Abstract | Publisher Full Text | Free Full Text

41. van der Zee HH, de Ruiter L, Boer J, *et al.*: **Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions.** *Br J Dermatol.* 2012; **166**(1): 98–106.
PubMed Abstract | Publisher Full Text

42. Ring HC, Thorsen J, Saunte DM, *et al.*: **The Follicular Skin Microbiome in Patients With Hidradenitis Suppurativa and Healthy Controls.** *JAMA Dermatol.* 2017; **153**(9): 897–905.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Guen-Revillet H, Jais JP, Ungeheuer MN, *et al.*: **The Microbiological Landscape of Anaerobic Infections in Hidradenitis Suppurativa: A Prospective Metagenomic Study.** *Clin Infec Dis.* 2017; **65**(2): 282–291.
PubMed Abstract | Publisher Full Text

44. Qiu F, Liang CL, Liu H, *et al.*: **Impacts of cigarette smoking on immune responsiveness: Up and down or upside down?** *Oncotarget.* 2017; **8**(1): 268–284.
PubMed Abstract | Publisher Full Text | Free Full Text

45. Kothari V, Galdo JA, Mathews ST: **Hypoglycemic agents and potential anti-inflammatory activity.** *J Inflamm Res.* 2016; **9**: 27–38.
PubMed Abstract | Publisher Full Text | Free Full Text

46. Richards JL, Yap YA, McLeod KH, *et al.*: **Dietary metabolites and the gut microbiota: an alternative approach to control inflammatory and autoimmune diseases.** *Clin Transl Immunology.* 2016; **5**(5): e82.
PubMed Abstract | Publisher Full Text | Free Full Text

47. Basson A, Trotter A, Rodruiguez-Palacios A, *et al.*: **Mucosal Interactions between Genetics, Diet, and Microbiome in Inflammatory Bowel Disease.** *Front Immunol.* 2016; **7**: 290.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Kramer CD, Genco CA: **Microbiota, Immune Subversion, and Chronic Inflammation.** *Front Immunol.* 2017; **8**: 255.
PubMed Abstract | Publisher Full Text | Free Full Text

49. Kim CH: **Immune regulation by microbiome metabolites.** *Immunology.* 2018; **154**(2): 220–229.
PubMed Abstract | Publisher Full Text | Free Full Text

50. Tan JK, McKenzie C, Marino E, *et al.*: **Metabolite-Sensing G Protein-Coupled Receptors-Facilitators of Diet-Related Immune Regulation.** *Annu Rev Immunol.* 2017; **35**: 371–402.
PubMed Abstract | Publisher Full Text

51. Frew JW, Vekic DA, Woods J, *et al.*: **A systematic review and critical evaluation of reported pathogenic sequence variants in hidradenitis suppurativa.** *Br J Dermatol.* 2017; **177**(4): 987–998.
PubMed Abstract | Publisher Full Text

52. Gooz M: **ADAM-17: the enzyme that does it all.** *Crit Rev Biochem Mol Biol.* 2010; **45**(2): 146–9.
PubMed Abstract | Publisher Full Text | Free Full Text

53. Blok JL, Li K, Brodmerkel C, *et al.*: **Gene expression profiling of skin and blood in hidradenitis suppurativa.** *Br J Dermatol.* 2016; **174**(6): 1392–4.
PubMed Abstract | Publisher Full Text

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Attikon University Hospital, Athens, Greece

This is a long time needed review trying to shed light in the pathogenesis of hidradenitis suppurativa (HS). My concerns are coming from the biggest hurdle the authors had to overcome from the very beginning of their attempt i.e. the great heterogeneity of the existing evidence. Due to this, I find over-exaggerated the conducted approach to set-up a mechanistic interpretation for the disease. I believe that the heterogeneity is so vast that it is almost impossible to suggest the pathways implicated in the pathogenesis of HS. To this end, I suggest that the mechanistic parts are omitted and Figure 2 as well.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology, genetics, hidradenitis, anti-cytokine therapies

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 February 2019

https://doi.org/10.5256/f1000research.18879.r43995

Aude Nassif
Institut Pasteur, Medical Center, Paris, France

This very instructive study aims at analyzing previous cytokine studies in HS patients, in skin tissue, blood, serum and exudates, to assess relevancy and reliability of these studies.

The authors have performed an extensive work, methods seem perfectly appropriate. The authors are very critical and rigorous in their approach, looking for confounding factors, which is highly desired.

The authors could also mention that genetic heterogeneity may play a role in the diversity of results and encourage using similar phenotypes for future studies.

This analysis brings up a very important and honest contribution to the current knowledge in cytokines involved in HS and therefore deserves indexing.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbiology, genetics, therapeutics, clinical forms of HS and associated diseases

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 07 February 2019

https://doi.org/10.5256/f1000research.18879.r43493

Barbara Horváth
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Lisette Prens
Department of Dermatology, University of Groningen, Groningen, The Netherlands

Thank you for the opportunity to review this manuscript and congratulations to the authors for their great efforts in putting this systematic review together. Research on the role of cytokines in HS is important, as it may lead to new targets for therapy and a better understanding of the pathophysiology of HS.

Summary

This systematic review focused on collecting all data published on cytokine studies in tissue, blood, serum and exudate in hidradenitis suppurativa. 81 discrete cytokines were examined in HS patients (n=564) and control patients (n=198) in 19 studies. Methodology varied greatly among studies, which were generally of low quality. When measuring levels of cytokines, substantial variance was found and the majority of cytokines showed heterogeneity. IL-17 signalling appeared to be a significant component. Suggestions for further research were discussed.

Questions

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes.

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes. However, I wonder why the term 'hidradenitis suppurativa' is not in the search strategy and 'hidradenitidis suppurative' is? 'Hidradenitidis' is not an existing word, as far as I know and will not provide any search results. Please adjust.

Is the statistical analysis and its interpretation appropriate?
Yes, as far as I can judge as a non-statistician. The analyses used are ones I have little experience with myself. I'll refrain from commenting on this section.

Are the conclusions drawn adequately supported by the results presented in the review?
Partly. The last conclusion 'examining the natural history of the disease through longitudinal measurements of cytokines over time' is not discussed anywhere else in this article. First, I suggest changing 'history' to 'course'. Moreover, I am wondering, how the authors propose to do this. Monitoring the natural course of the disease, would mean patients cannot receive any treatment for their HS, during this proposed study. Depending on how long the natural course is meant to be monitored, I don't think it is ethical to withhold patients from treatment.
Please elaborate on this conclusion with a specific proposal or otherwise rephrase or maybe leave out this conclusion.

Other comments
Page 9 last paragraph/Page 28 – 1st paragraph: You state that 'psoriasiform epidermal hyperplasia is seen in HS'. Please provide a reference for this statement. The reference provided only references to the pathway likely responsible for this in psoriasis.

Page 28 – 4th paragraph: 'These potential mechanistic pathways (which may prime or contribute towards

inflammation in HS) require validation in functional studies.' Could you please provide an example on how such a functional study should be designed to produce reliable results?

Table 4: the abbreviation 'Lpa' is not clarified in the key section of the table. Does 'Le' (page 11, IL-1a, first row) mean lesion exudate?

Table 6: the number four of question four is missing in the top row of the table on both pages (24-25). Please insert.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hidradenitis suppurativa

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

4.1.2: Publication 1-2

Frew JW, Hakes JE, Krueger JG "A Systematic Review and Critical Evaluation of Immunohistochemical Associations in Hidradenitis Suppurativa" F1000Research (2018); 2018, 7:1923

SYSTEMATIC REVIEW

REVISED A systematic review and critical evaluation of immunohistochemical associations in hidradenitis suppurativa [version 2; peer review: 2 approved]

John W. Frew, Jason E. Hawkes, James G. Krueger

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v2 **First published:** 11 Dec 2018, **7**:1923 (https://doi.org/10.12688/f1000research.17268.1)
Latest published: 17 Jun 2019, **7**:1923 (https://doi.org/10.12688/f1000research.17268.2)

Abstract

Background: Hidradenitis suppurativa (HS) is a chronic inflammatory disease with significant morbidity and impact on quality of life. Our understanding of the pathophysiology is incomplete, impairing efforts to develop novel therapeutic targets. Immunohistochemistry studies have produced conflicting results and no systematic evaluation of study methods and results has been undertaken to date.
Methods: This systematic review aimed to collate and describe all reports of immunohistochemical staining in HS. This systematic review was registered with PROSPERO and conducted in line with the PRISMA reporting guidelines. Potential bias was assessed using the NIH Criteria and antibodies used across various studies were tabulated and compared.
Results: A total of 22 articles were identified describing results from 494 HS patients and 168 controls. 87 unique immunohistochemical targets were identified. The overall quality of studies was sub-optimal with staining intensity confounded by active treatment. Conflicting data was identified and able to be reconciled through critical evaluation of the study methodology.
Conclusions: Keratinocyte hyperplasia with loss of cytokeratin markers co-localizes with inflammation comprising of dendritic Cells, T-lymphocytes and macrophages, which are known to play central roles in inflammation in HS. Primary follicular occlusion as a pathogenic paradigm and the principal driver of HS is unclear based upon the findings of this review. Inflammation as a primary driver of disease with secondary hyperkeratosis and follicular occlusion is more consistent with the current published data.

Keywords

Hidradenitis Suppurativa, Cytokeratin, Immunohistochemistry, Pathogenesis, Inflammation, Follicular Occlusion

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
REVISED **version 2** published 17 Jun 2019		✓ report
version 1 published 11 Dec 2018	✓ report	? report

1 **Gregor B. E. Jemec**, University of Copenhagen, Copenhagen, Denmark

2 **Martin M. Okun**, Fort HealthCare, Fort Atkinson, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: John W. Frew (jwfrew@gmail.com)

Author roles: Frew JW: Conceptualization, Data Curation, Formal Analysis, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Hawkes JE**: Data Curation, Formal Analysis, Methodology, Writing – Review & Editing; **Krueger JG**: Formal Analysis, Supervision, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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First published: 11 Dec 2018, **7**:1923 (https://doi.org/10.12688/f1000research.17268.1)

REVISED **Amendments from Version 1**

Based upon the comments of reviewers the following changes have been made:

1. The conclusions which previously read: *"…primary follicular occlusion as a pathogenic paradigm and the principal driver of HS is not consistent with the findings of this review…"*, has been altered to now read: *"…primary follicular occlusion as a pathogenic paradigm and the principal driver of HS is unclear based upon the findings of this review…"*
2. Minor points raised including the sentence which read: *"Increased K16 expression was increased...."*, has now been corrected to read: *"K16 expression was increased...."*

See referee reports

Introduction

Hidradenitis suppurativa (HS) is a chronic inflammatory disease, the exact pathophysiology of which remains incompletely defined[1]. Numerous inflammatory mediators including TNF-α[2], IL-17[2,3], IL-32[4] and IL-36 subtypes[5,6] have been implicated in the disease. However, there is an incomplete understanding of the source and triggers of these mediators and how they sustain the chronic inflammation that characterizes this disease[1,2]. The pathogenic paradigm of HS has evolved dramatically since the first description by Velpau in 1839[7]. First thought of as an apocrinitis of infectious aetiology, it is now considered a disorder of follicular occlusion and more recently an inflammatory disease characterised by a keratinocyte mediated inflammatory response[6]. However, the variable response to topical, systemic and biologic therapies in HS[8] indicate our understanding of disease pathophysiology is incomplete when compared to other cutaneous inflammatory diseases such as psoriasis[9] and atopic dermatitis[10]. Existing studies examining the histology and immunohistochemical profiling of HS tissues represent conflicting results, for example in the degree of dermal dendritic cell infiltration[11,12] and the production of TNF-alpha in the follicular unit[13,14]. These results may be influenced by heterogeneous sampling methods, laboratory processing methods and data analysis[15]. An additional complicating factor is that clinical comorbidities which are strongly associated with disease activity in HS, such as obesity[16], diabetes[17], inflammatory bowel disease[18], and smoking[19] also impact inflammatory cell activity in the skin[18,20–22]. Hence it remains unclear whether the presence or absence of these conditions may confound the findings of immunohistochemical studies in HS[15,23] and whether clinical stratification of patients is required to identify distinct pathogenic pathways, which may be amenable to pharmacological intervention. This variability across studies makes comparing data problematic. To date no systematic analysis of immunohistochemical studies has been undertaken to compare results, methodology and analytical techniques.

Objectives

The objectives of this systematic review are:

1) To collate and describe all published reports of immunohistochemical studies in HS

2) To critically evaluate the sampling, laboratory and analysis techniques used in each study to determine if comparisons can be made across studies.

Methods

This systematic review was registered with PROSPERO[24] (Registration number CRD42018104763) and was conducted in line with the PRISMA[25]. The STROBE statement[26] was used to assess the observational studies included in this study.

Data sources

Information Sources for this review encompassed Pubmed (1946-July 1 2018), Scopus (2004- July 1 2018) and Web of Science (1990-July 1 2018) as shown in Figure 1. Search strategy is presented as Table 1.

Study eligibility criteria

Eligibility criteria for this review included cohort studies, case-control studies and other observational studies with no restrictions of patient age, sex, ethnicity or language of publication. Eligible studies included those reporting the results of immunohistochemical findings in HS. Studies deemed not eligible included articles which provided no new data, only a review or summary of previously published data.

Appraisal and synthesis methods

Data collection was performed independently by 2 authors (JWF & JEH), with any disagreements regarding inclusion of citations being referred to a third author (JGK) for mediation. Information was collected using a standardized data collection form (available as Extended data[27]) with the principal outcomes of interest being the immunohistochemical stain of interest, the site and rated intensity of staining (as described by authors), and comparison with perilesional/ unaffected/ control tissue. If data from individual patients was not available then the aggregate data was collected.

Potential sources of bias in the identified studies are acknowledged including the small size of patient cohorts, the variability in sampling and laboratory techniques, antibodies published and reactants used. Therefore these variables (where available) were collated to assess the heterogeneity of studies. Bias was also assessed using the NIH quality assessment tool for observational studies[28].

Results

A total of 425 non-duplicated citations were identified in the literature review (Figure 1). 398 of these articles were removed upon review of titles and abstracts against the pre-defined eligibility criteria. Full text review of the remaining 27 articles excluded 5 articles providing no new data. The remaining 22 studies[4–6,11–14,29–43] reporting the results of 494 individual HS patients and 168 control patients were used as the basis of this systematic review.

Descriptive analysis

The demographics of the patients of the included studies are presented in Table 2. Of 494 HS patients, 180 were male (38.3%) and 290 female (61.7%) with 24 cases unreported. Ages ranged from 15–72 years. 47/50 (94%) of reported cases were smokers, 12/30 (40%) had a BMI >30, and there was no information pertaining to diabetes or family history of HS. Of the 200 documented biopsy sites 93 were axillae (46.5%), 69 were inguinal (34.5%), and 38 were genital (19%) (Table 3). 64 patients

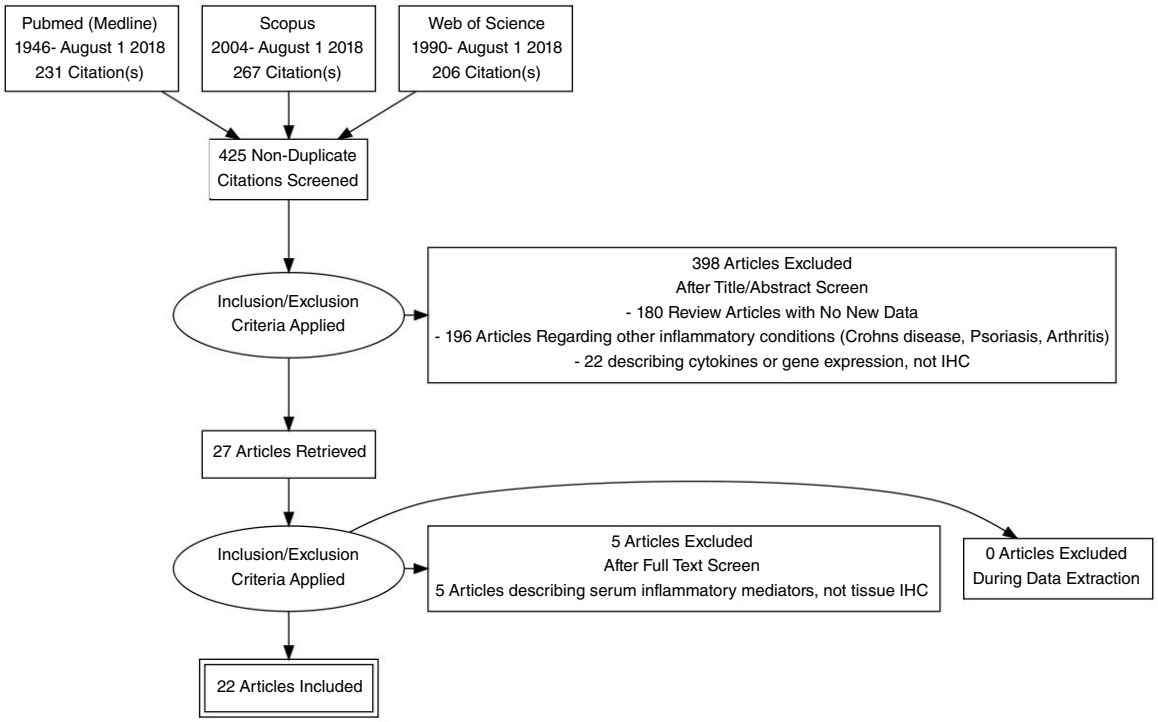

Figure 1. PRISMA flowchart.

Table 1. Search Strategy for Systematic Review Entitled *"A Systematic Review and Critical Evaluation of Immunohistochemisty Studies in Hidradenitis Suppurativa*.

Resources:	
	1) Pubmed (1946-July 1 2018), 2) Scopus (2004- July 1 2018) 3) Web of Science (1990-July 1 2018) 4) Published Abstracts 5) Contact with Authors for abstracts without full text for clarification of data and methodology
Pubmed Search Strategy:	
	acne inversa OR apocrine acne OR apocrinitis OR Fox-den disease OR hidradenitis axillaris OR HS OR pyodermia sinifica fistulans OR Velpeau's disease OR Verneuil's disease OR Hidradenitidis Suppurative AND IHC OR Immunohistochemistry OR Histology

had Hurley staging with 7/64 (10.9%) Stage 1, 40/64 (62.5%) Stage 2 and 17/64 (26.6%) Stage 3. No individual Sartorius scores were reported. Where current treatment was reported, 6 patients (4.5%) were on adalimumab, 42 (31.6%) were untreated, 85 patients (63.9%) had treatment withheld prior to biopsy, and 357 cases were unreported. Lesional biopsies were taken from all studies, with 3 individual studies also taking perilesional biopsies[6,30,41]. Age and Sex matched controls were present in 3 studies[29,31,43] and results were stratified in a minority of studies. 2 studies stratified by disease severity[4,12], 7 studies stratified by lesion site[13,33–37,39], 5 studies stratified by treatment[4,5,12,29,32], and no studies stratified by comorbidities. Analysis of immunohistochemical staining methodology varied and included quantitative analysis (3 studies)[14,30,32], semi-quantitative analysis (14 studies)[4,5,11–13,29,34,37–43], and the presence or absence of staining (5 studies)[6,31,33,35,36]. A total of 87 distinct immunohistochemical staining targets were identified (Table 4, Table 5 and Table 6).

Immunohistochemistry results

Epidermis. The epidermis of HS lesional tissue expressed the normal array of keratins (K) in the basal (K5, K14) and suprabasal (K1, K2e, K10) layers. K6, K16 and K17 staining were increased compared to healthy controls in the suprabasal epidermis in one study[30], however, K6 and K17 staining was not increased in the epidermis (only in non-keratinized portions of sinus tracts) in a second study[38]. Where K6 and K17 were positive in suprabasal epidermis, K17 staining was more pronounced than K6 staining[30]. K19 was weakly positive in acanthotic epidermis[37]. Ki67 staining was elevated in basal and suprabasal epidermis. Normal staining patterns of desmoplakin, plakophilin and plakoglobin were seen[38]. Cells staining positive for CD1a, CD206, CD207 and CD209 were seen throughout the epidermis[40]. CD3, CD4, CD8 and to a lesser degree CD68 positive cells demonstrated epidermotropism in sites of epidermal acanthosis[30,33]. CD29 and cholera toxin (double positive)

Table 2. Demographic data of included studies.

Number of HS Patients	Male	Female	Mean Age (Years)	Comorbidities				Biopsy Sites			Hurley Staging	mHSS Score (Mean)	Therapy	Study Reference
				Smoking	Obesity (BMI>30)	Diabetes	Family History	Axillae	Groin	Genital				
18	11	7	(Range 19–62)	NR	NR	NR	NR				NR	NR	NR	14
15	6	9	38.7	NR	NR	NR	NR	9	4	2	Stage 1=0 Stage 2=10 Stage 3=5	NR	Nil	6
24	8	16	36.5 (range 21–51)	NR	NR	NR	NR	NR	NR	NR	Mean=2.29 (SD=0.62)	NR	Untreated	29
22	10	12	38.2 (Range 19–60)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	30
10	5	5	42 (Range 21–49)	NR	NR	NR	NR	Y	Y	N	Stage 2 (100%)	NR	Treatment Withheld	32
20	8	12	37.5 (Range 21–51)	N=18	N=10	NR	NR	NR	NR	NR	NR	NR	Treatment Withheld (8 weeks)	4
25	9	16	36 (Range 18–51)	NR	NR	NR	NR	NR	NR	NR	Mean =2.16 (SD=0.55)	NR	Treatment Withheld (3 weeks)	5
47	19	28	42.3 (Range 22–54)	NR				NR	NR	NR		48.3 (Range 8–144)	NR	31
11	9	2	39.6 (Range 18–61)	NR	NR	NR	NR	NR	NR	NR	"Mod-Severe Disease"	NR	NR	
20	6	14	40 (SD=15)	19	27.6 (4.1)	NR	NR	7	12	1	Stage 1=4 Stage 2=11 Stage 3=5	NR	Treatment withheld 3 weeks prior	12
10	1	9	38 (SD=15)	10	28.9 (SD 4.5)	NR	NR	3	7	0	Stage1=2 Stage2=7 Stage3=1	NR	Treatment Withheld 3 weeks prior	
14		1	30	NR	NR	NR	NR	1		0	NR	NR	NR	13
		1	42	NR	NR	NR	NR		1		NR	NR	NR	
		1	25	NR	NR	NR	NR		1		NR	NR	NR	
		1	22	NR	NR	NR	NR	1			NR	NR	NR	
		1	45	NR	NR	NR	NR		1		NR	NR	NR	
		1	27	NR	NR	NR	NR	1			NR	NR	NR	
	1		38	NR	NR	NR	NR	1			NR	NR	NR	
	1		34	NR	NR	NR	NR	1			NR	NR	NR	
		1	59	NR	NR	NR	NR		1		NR	NR	NR	
		1	41	NR	NR	NR	NR	1			NR	NR	NR	
		1	33	NR	NR	NR	NR	1			NR	NR	NR	
		1	46	NR	NR	NR	NR		1		NR	NR	NR	
		1	49	NR	NR	NR	NR		1		NR	NR	NR	
	1		31	NR	NR	NR	NR		1		NR	NR	NR	
60	26	34	37.3 (Range 15–67)	NR	NR	NR	NR	1	6	1	NR	NR	NR	33

Number of HS Patients	Male	Female	Mean Age (Years)	Comorbidities				Biopsy Sites			Hurley Staging	mHSS Score (Mean)	Therapy	Study Reference
				Smoking	Obesity (BMI>30)	Diabetes	Family History	Axillae	Groin	Genital				
9	1		47	NR	Y	NR	NR		1		3	NR	adalimumab	11
		1	31	NR	N	NR	NR		1		1	NR	adalimumab	
	1		24	NR	N	NR	NR	1			3	NR	adalimumab	
		1	32	NR	N	NR	NR			1	3	NR	adalimumab	
		1	58	NR	N	NR	NR			1	3	NR	adalimumab	
	1		58	NR	N	NR	NR			1	2	NR	adalimumab	
	1		36	NR	Y	NR	NR		1		2	NR	Nil	
	1		39	NR	N	NR	NR		1		3	NR	Nil	
	1		67	NR	N	NR	NR			1	3	NR	Nil	
16	1	15	NR	NR	N	NR	NR	3		13	NR	NR	NR	34
5	1	4	18-36	NR	NR	NR	NR	2	3		NR	NR	NR	35
50	18	32	11-70	NR	NR	NR	NR	39	6	5	NR	NR	NR	36
14	11	3	16-72	NR	NR	NR	NR	2	12	0	NR	NR	NR	37
15	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	38
22	6	16	45.6 (Range 29–69)	NR	NR	NR	NR	13	7	2	NR	NR	NR	39
9	3	6	44 (Range 32–70)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	40
12	0	12	29.4 (Range 19–42)	NR	NR	NR	NR	3	0	9	NR	NR	NR	41
36	13	23	25 (Range 20–69)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	42
10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	43
494	**180**	**290**		**47/50 Reported**	**12/30 Reported**	**None Reported**	**None Reported**	**93/200**	**69/200**	**38/200**	**Hurley 1= 7 Hurley 2= 40 Hurley 3= 17 Unknown= 430**	**No Individual Scores Reported**	**adalimumab=6, untreated=42, treatment withheld=- 85, not reported= 357**	

BMI= Body Mass Index mHSS= modified Hidradenitis Suppurativa Score (Sartorius Score) NR= Not Reported

F1000Research 2019, 7:1923 Last updated: 25 JUN 2019

Table 3. Critical Evaluation of Methodology of Studies Included in This Review.

IHC Targets	Number of HS Patients	Number of Controls	Samples Analyzed	Age/Sex Matched Controls	Stratified by severity	Stratified by lesion site	Stratified by Co-morbidities	Stratified by Treatment	Immunostaining Intensity Assessment	Study Reference
α-MSH, LL37, S100A7, MIF, TNF-α, hBD3, lysozyme	18	12	L	N	NR	N	N	N	Quantitiative Immunohistomorphometry (Image J Software)	14
IL36	15	15	L, PL	NR	NR	N	N	N	Present/ Absent	6
CD3, CD56 LL37	24	9	L	Y	NR	NR	N	Y (untreated)	Semiquantitative (0-3)	29
CD1a, CD4, CD8 CD20, CD56, Factor XIIIa, IL17, NLRP3, Caspase-1	22	Yes (NR)	L, PL, U, C	NR	N	N	N	N	Cell Counting square grid x400 magnification	30
IL-23, IL-12, CD68, CD4	10	8	L, C	N	N	N	N	Y (ceased 3/52 prior)	Positive stained cells per mm^2	32
IL-32	20	10	L, C, S	N	Y	N	N	Y (ceased 8/52 prior)	Semiquantitative (+ to ++++)	4
IL-36	25	7	L, C, S	N	N	N	N	Y (ceased 3/25 prior)	Semiquantitative (+ to ++++)	5
LCN2	10	16	L	Y	N	N	N	N	Present/ Absent	31
CD11c	20	6	L	N	Y	N	N	Y	Semiquantitative (+ to ++++)	12
MMP2 hBD2 TNF-α	14	2	L, C	N	N	Y	N	N	Semiquantitative ((+ to ++++)	13
CD3, CD4, CD8, CD68 CD79 CD56	60	Yes (NR)	L,C	N	N	Y	N	N	Present or Absent	33
CD3, CD4, CD8, CD20, CD138, CD14, CD68, CD11c	9	Yes (NR)	L,C	N	N	N	N	N	Semiquantitative (+ to ++++)	11
GCDFP-15, CD15, Lysosyme, S100, Ca19-9, HMB45	13	3	L,C	N	N	Y	N	N	Semiquantitative (+ to ++++)	34
CD29, CTx-FITC	5	4	L,C	N	N	Y	N	N	Present or Absent	35
AE1/AE3/PKC26/ Enhanced Alkaline Phosphatase	50	Y (NR)	L,C	N	N	Y	N	N	Present or Absent	36
K1, K10, K14, K16,k17, K19,	14	1	L,C	N	N	Y	N	N	Semiquantitative (+ to ++++)	37
Desmoplakin 1,2, Plakoglobin, Plakophilin 1,2, Desmoglein 1,2,3, Desmocollin 1,2,3,K2e, K4, K5, K6, K7, CK8,CK9, CK10, K13, K13/15/16, K14, K17,K19, K20 Ki67	15	Y (NR)	L,C	N	N	N	N	N	Semiquantitative (+ to ++++)	38
ER, AR	22	10	L,C	N	N	Y	N	N	Semiquantitative (+ to ++++)	39
TLR2, CD3, CD19, CD56,CD68, CD11c, CD1a, CD206, CD207, CD209	9	Y (NR)	L,C	N	N	N	N	N	Semiquantitative (+ to ++++)	40
TLR 2,3,4,7,9,ICAM-1, TNF-α, IL-6, IL-10, TGF-β, α-MSH, hBD2, hBD4 IGF-1	12	Y (NR)	L,PL	N	N	N	N	N	Semiquantitative (+ to ++++) Epidermis Only	41
hBD3, S100A7, RNase7	36	57	L,C	N	N	N	N	N	Semiquantitative (+ to ++++)	42
MMP8	10	8	L,C	Y	N	N	N	N	Semiquantitative (+ to ++++)	43
	494	168		**3/22**	**2/22**	**7/22**	**0/22**	**5/22**		

Table 3: Critical Evaluation of Methodology of Studies Included in This Review Key:L= Lesional, PL= Perilesional, U= Uninvolved, C= Control S=Serum, Y=Yes, N=No, NR= Not Reported, CTx-FITC =Cholera Toxin

F1000Research 2019, 7:1923 Last updated: 25 JUN 2019

Table 4. Data pertaining to distribution of cells expressing Immunohistochemical markers described in this review.

Cell Type	Study	Results
Basal Keratinocytes	13	MMP2 Expressed
Suprabasal Keratinocytes	31	LCN 2 staining of suprabasal keratinocuytes
Dermal Fibroblasts	13	MMP2 expressed
	7	33/51 specimens associated with ++ fibrosis
Neutrophils	13	MMP2 in keratinocytes, fibroblasts, macrophages and lymphocytes,
	30	Significant increase in number of neutrophils in dermis Dermis> Perifollicular
	31	LCN2 in neutrophils – epidermis and dermis
	7	+++ infiltrate in 29/51 specimens
Plasma Cells	7	+++ Plasma cell infiltrate in 2/51 specimens
Eosinophils	7	+++ Eosinophilic infiltrate ++ in 3/51 specimens
Histiocytes	7	+++ Infiltrate 24/51 speciments
Lymphocytes (NOS)	13	MMP2 expressed TNF alpha positivity in dermis
	44	Lymphocytes, giant cell and necrosis in established lesions
T Cells	4	CD3 + Dermis producing IL32 CD 56 + NK T cells producing IL32
	33	lymphocytic mixed infiltrate perifollicular (with unruptured terminal follicles). This consisted of CD-3 (39%), CD-4 (30%), CD-8 (14%), and positive cells (CD-4/CD-8 ratio: 2.1:1). CD-56 (0.1%) and UCHL-1 (0%) brought no conclusive results. Conspicuous was a CD-8 cell positive folliculotropism in all immuno- histologies (Figure 3). CD-8 positive lymphocytes were loosely distributed not only in the stratum basale but also in the suprabasal epithelial areas. The subepidermal inflammatory infiltrate in the area of interfollicular epidermal hyperplasia showed a comparable cellular composition: CD-3 (38%), CD-4 (26%), CD-8 (19%), CD-56 (0.2%) and UCHL-1 (0%), CD-4/CD-8 Ratio: 1.4:1. Here too, a CD-8 positive pronounced epidermotropism was impressive
	30	At perifollicular sites, quantitative analysis showed a significant increase in the mean number of CD3+, CD4+ and CD8+ T lymphocytes (CD3+, 34 ` 20 per HPF; CD4+, 38 ` 21; CD8+, 12 ` 8) compared with healthy control skin (CD3+, 9`4; CD4+, 2`1; CD8+, 1`1;
	3	CD4 T cells producing IL17 in dermis
	32	CD4 T cells producing IL17 in dermis
B Cells	11,12	Psuedolymphomatous nests (see cytokine studies)
	33	Perifollicular infiltrate with unruptured terminal follicles: CD-79 (35%) Subepidermal interfollicular Infiltrate: CD-79 (33%),
Dendritic Cells	11	Successful Adalimumab treatment reduced influx of CD11c+ dendritic cells in lesional skin
	12	Number of dendritic cells stable in skin- mild elevation only
	4	Dermis producing IL32
Macrophages	13	MMP2 expressed TNF alpha positivity
	30	Significant increase in deep infiltrate
	32	Increase with co-staining of CD68/CD32 and IL12/ IL23
	33	Perifollicular infiltrate with unruptured terminal follicles: CD-68 (12%) Subepidermal interfollicular Infiltrate: CD-68 (19%),
	4	Dermis producing IL32
Mast Cells	30	Significant increase in deep infiltrate
	12	Significant increase in deep infiltrate

Table 5. Reported Immunohistochemical Staining Results Identified in this Systematic Review.

IHC Staining Target	Epidermis		Dermis	Hair Follicles		Sinus Tracts				Subcutis	Apocrine/ Eccrine Glands	Study Reference
	Suprabasal Staining	Basal Staining	Dermal Staining	Infundibular Staining	ORS Staining	Type 1		Type 2	Type 3			
						Type A	Type B	Type C				
CD1a			+									30
	++		+									40
			+++									12
CD3	++		++		++							30
	+	+	+									40
												32
CD4			++									12
			Interfollicular and perifollicular									33
			+		+							30
												32
CD8			+									12
	Epidermotropism		Interfollicular and perifollicular									33
			+		+							30
CD11c		+++										12
			+++									40
CD14												12
CD15											+	34
CD19	-		+									40
CD20			+++									30
			+++									12
CD29	+	+		+		+ (NOS)						35
CD32												32
CD56												30
	-		+									40
CD68	Deep> Perifollicular		++		+							30
			Interfollicular and perifollicular									33
	+		+++									40
												32
CD79			Interfollicular and perifollicular									33
CD138			Mild infiltrate									12
CD206	+		+++									40

IHC Staining Target	Epidermis		Dermis	Hair Follicles		Sinus Tracts				Subcutis	Apocrine/ Eccrine Glands	Study Reference
	Suprabasal Staining	Basal Staining	Dermal Staining	Infundibular Staining	ORS Staining	Type 1		Type 2	Type 3			
						Type A	Type B	Type C				
CD207	+++*		+									40
CD209	++		++++									40
Cytokeratins												
AE1			Single K									36
AE3			Single K									36
PKC26			Single K									36
Factor XIIIa			DC +									12
			+		+							30
K1	Present in acanthotic epidermis			+	-	+	+	-			-	37
K2e	++					-		-	-			38
K4	-					-		-	-			38
K5	+					+		+	+			38
K6	-					+		+	+			38
K5/6§	++++											30
K7	-					-		-	+			7
K8	-					-		-	-			38
K9	-					-		-	-			38
K10											-	36
	Present in acanthotic Epidermis			+	-	+	+	-			-	37
	++	+				++		-	-			38
K13	-					+		+	-			38
K13+15+16§	+	+				+		+	+			38
K14	Highly positive in acanthotic epidermis			+	+	+	+	++			Sebaceous Duct and Gland +	37
						+		+	+			38
K15											Stained apocrine glands	34
K16	Weakly positive in acanthotic epidermis			-	+	-	+	+			-	37
K17	Weakly positive in acanthotic epidermis			-	+	-	+	+			-	37
K18												38

IHC Staining Target	Epidermis		Dermis	Hair Follicles		Sinus Tracts				Subcutis	Apocrine/ Eccrine Glands	Study Reference
	Suprabasal Staining	Basal Staining	Dermal Staining	Infundibular Staining	ORS Staining	Type 1		Type 2	Type 3			
						Type A	Type B	Type C				
K19						+ NOS					+	36
	Weakly positive in acanthotic epidermis			-	+	-	-	-			-	37
						-		-	++			38
K20						-		-	-			38
Ki67	+					++		++	++			38
ER	-										+	39
AR	-										NC	39
GCDFP-15											Apocrine glands	34
S100											Eccrine glands	34
Lysozyme											Vulval cases only	34
		↓ in scarred cases										14
HMB45	-										Negative all cases	34
TLR2	++		++++									40
	↓											41
TLR3	↓											41
TLR4	↓											41
TLR7	↓											41
TLR9	↓											41
ICAM-1	↓											41
TGF-β	↓											41
IGF-1	↓											41
RNase7	+++											42
MMP2	+++/++++	+++/++++	+		+++	+ NOS						13
MMP8	(Neutrophils)		+++			(Neutrophils)						43
Cholera Toxin	Slopes of papillae suprabasal epidermis,			hair follicles		+ NOS						35
Desmoplakin 1	++					++		++	+			38
Desmoplakin 2	++					++		++	+			38
Plakoglobin	++					++		++	+			38

IHC Staining Target	Epidermis		Dermis	Hair Follicles		Sinus Tracts				Subcutis	Apocrine/ Eccrine Glands	Study Reference
	Suprabasal Staining	Basal Staining	Dermal Staining	Infundibular Staining	ORS Staining	Type 1		Type 2	Type 3			
						Type A	Type B	Type C				
Plakophilin 1	++					++		++	+			38
Plakophilin 2						-		-	-			38
Desmoglein 1	++	+				++		++	-			38
Desmoglein 2	+					+		++	++			38
Desmoglein 3	++					++		++	+			38
Desmocollin 1	++	+				++		-	-			38
Desmocollin 2	++	+				++		++	+			38
Desmocollin 3	++					++		++	+			38
hBD2	↓										Negative in 12/14	1
	↓											41
hBD3	++ (suprabasal)	-			++							14
	+++	+										42
hBD4	↓											41
TNF-α	++/+++ (macrophage/ lymphocytes)				++/+++						+++	13
	++	++	+	NC	↓							14
IL-6	↓											41
IL-10	↓											41
IL-12			++++									32
IL-23			++++									32
IL-17			Diffuse									30
			+++									32
IL-32		++	+++									4
IL-36	+	+++										6
	Suprabasal	+++										5
Caspase1	++											30
NLRP3	++											30
MIF	++				++							14
S100A7	++	+++			++							14
	++											42
LL-37	++				++						NC	14
		++	+++		++							29
α -MSH	++				NC							14

Key: + to ++++ = Degree of positive staining, - = reported negative staining, NC= No Change; ↓ Decreased, NOS= Not Otherwise Specified, *= Statistically significant result compared with healthy controls, §=Pan Cytokeratin Stain, DC= Dendritic Cells, Single K= Single keratinocytes,

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Table 6. Immunohistochemistry stains/antibodies used in included reviews.

Target	Details	Study Reference
CD1a	CloneO10; Dako Cytomartion	30
	CloneO10; Dako Cytomartion	40
	O10 1:20 Immmunotech, Prague, Czech Republic	12
CD3	clone F7.2.38; Dako)	30
	Polyclonal rabbit anti-human CD-3 dilution 1:25; Dako Cytomation Denmark A/S, Glostrup, Denmark),	33
	Polyclonal 1:150 Dako, Glostrup, Denmark	12
	Clone PC3/188A; DakoCytomation, Glostrup, Denmark	40
CD4	4B12 1:160 Monosan Uden The Netherlands	12
	monoclonal mouse anti-human CD-4 dilution 1:10; Vision Biosystems Novocastra, Newcastle, UK	33
	clone 4B12; Dako	30
	MT310 Dako	32
CD8	C9/144B 1:100 Dako	12
	monoclonal mouse anti-human CD-8 dilution 1:50; Dako Cytomation Denmark A/S	33
	clone C8/144B; Dako	30
CD11c	5D11 1:60 Novocastra Newcastle Upon Tyne, UK	12
	Clone KB90 DakoCytomation	40
CD14	MY4 1:100 Novocastra Newcastle Upon Tyne, UK	12
CD15	Not Reported	34
CD19	Clone HD37; DakoCytomation	40
CD20	clone L26 (1,4); Dako	30
	L 26 1:400 Dako	12
CD29	fluorescein-tagged B-subunit of cholera toxin (CTx-FITC) + CyChrome (Pharmingen BD Biosciences, Franklin Lakes, NJ, USA)	35
CD32	KB61 Dako	32
CD56	clone 123C3; Dako	30
	Clone MOC-1; DakoCyotmation	40
	monoclonal mouse anti-human CD-56 1:50; Dako Cytomation Denmark A/S),	33
	123C3.D5 1:25 Thermo Fisher Scientific Altrincham UK	12
CD68	Clone PG- M1; Dako)	30
	monoclonal mouse anti-human CD-68 dilution 1:50; Dako Cytomation Denmark A/S)	33
	Clone EBM11; Dako Cytomation	40
	KP1 1:160 Dako	12
	EBM11 Dako	32
CD79a	monoclonal mouse anti-human CD-79 dilution 1:25; Dako Cytomation Denmark A/S)	33
	JCB117 1:100 Dako	12

Target	Details	Study Reference
CD138	B-A38 1:25 IQ Products Groningen, The Netherlands	12
CD206	Clone 19.2; BD Biosciences Pharmingen	40
CD207	Clone DCGM4; Immunotech, Marseilles, France	40
CD209	Clone DCN46; BD Biosciences Pharmingen, San Diego Ca USA	40
Cytokeratins		
Pankeratin	AE1/AE3/PKC26; Ventana Medical Systems SA, Illkirch, Cedex, France	36
	AE1/AE3 1:200 Thermo Fisher Scientific	12
Factor XIIIa	AC-1A1 1:200 Thermo Fisher Scientific	12
	clone E980.1; Leica Biosystems Newcastle, Newcastle upon Tyne, U.K.)	30
K1	34 Beta B4 Novo Castra Laboratories Ltd, Newcastle- upon-Tyne, UK)	37
K2e	Ks2´342´ 7.1 against CK 2e (Dr L.Langbein, Heidelberg, Germany),	38
K4	6B10 against CK 4,	38
K5	AE 14 against CK 5,	38
K6	Ks6.KA12 against CK 6,	38
K5/6	clone M7237; Dako	30
CK7	OV-TL 12/30 and Ks7´18 against CK 7,	38
K8	CAM 5´2 against CK 8,	38
K9	HK9TY1 (guinea-pig polyclonal) against CK 9 (Dr L.Langbein)	38
K10	Not Reported	36
	LHP1 Novo Castra Laboratories Ltd, Newcastle- upon-Tyne, UK)	37
	MoAbs K8´60 and DE-K10 against CK 10,	38
K13	Ks13´1 against CK 13,	38
K13+15+16	Ks8´12 against CK 13 15 16,	38
K14	LL001 Novo Castra Laboratories Ltd, Newcastle- upon-Tyne, UK)	37
	LL001 against CK 14,	38
K15	Not Reported	34
K16	LL025 Novo Castra Laboratories Ltd, Newcastle- upon-Tyne, UK)	37
K17	E3 Novo Castra Laboratories Ltd, Newcastle- upon-Tyne, UK)	37
	Ks17.E3 against CK 17,	38
K19	Not reported	36
	B170 Novo Castra Laboratories Ltd, Newcastle- upon-Tyne, UK)	37
	Ks19´1 against CK 19,	38
K20	IT-Ks20´10 against CK 20	38
Ki67	MIB 1 against Ki-67	38
	MIB1 1:100 Dako	12

Target	Details	Study Reference
ER	ER (Thermo Scientific; pretreatment EDTA, pH 9.0, dilution 1:80).	39
AR	AR (Santa Cruz; pretreatment citrate, pH 6.0, dilution 1:100)	39
GCDFP-15	Not Reported	34
S100	Not Reported	34
Lysozyme	Not Reported	34
	A0099 pAbG 1:100 rabbit antihuman Dako Corporation	14
HMB45	Not Reported	34
TLR2	Clone TL2.3; Alexis Corp. San Diego Ca USA	40
	Santa Cruz Biotechnology, Inc, Santa Cruz, California	41
TLR3	Santa Cruz Biotechnology, Inc, Santa Cruz, California	41
TLR4	Santa Cruz Biotechnology, Inc, Santa Cruz, California	41
TLR7	Santa Cruz Biotechnology, Inc, Santa Cruz, California	41
TLR9	Santa Cruz Biotechnology, Inc, Santa Cruz, California	41
ICAM-1	Beckman Coulter, Inc, Brea, California	41
TGF-β	AbD Serotec	41
IGF-1	R&D Systems, Inc, Lille, France	41
RNase7	Dako	42
MMP2	MMP-2 (cat no. AF902, LOT DUB034081, obtained from goat, 1:100 dilution, R&D Systems	13
MMP8	Dako	43
Cholera Toxin	fluorescein-tagged B-subunit of cholera toxin (CTx-FITC) + CyChrome (Pharmingen BD Biosciences, Franklin Lakes, NJ, USA)	35
Desmoplakin 1	DP 1 2±2´ 15 and DP 1±2´ 17 against DP I II,	38
Desmoplakin 2	DP 1 2±2´ 15 and DP 1±2´ 17 against DP I II,	38
Plakoglobin	PG 5´ 1 and PG 11E4 (Dr M.J.Wheelock, Toledo, OH, U.S.A.) against PG,	38
Plakophilin 1	PP1-9E7 and PP1-5C2 against PP 1,	38
Plakophilin 2	PP2- 150 against PP 2,	38
Desmoglein 1	Dsg1E-P124 and Dsg1E-P23 against Dsg1,	38
Desmoglein 2	Dsg2E-G129 and Dsg2E- G96 against Dsg2,	38
Desmoglein 3	Dsg3-G194 and 5G11 against Dsg3,	38
Desmocollin 1	Dsc1-U100 against Dsc1,	38
Desmocollin 2	DC-Rab 36 (rabbit polyclonal) against Dsc2,	38
Desmocollin 3	MoAb Dsc3-U114 against Dsc3,	38

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Target	Details	Study Reference
hBD2	Human beta-defensin 2 (cat no. AF 2758, LOT VJU015051, obtained from goat, 1:100 dilution, R&D Systems, Germany)	13
	Abcam, San Francisco, California	41
hBD3	1:400 rabbit antihuman Donated by Prof Schroders Labor Kiel germany	14
	1:1000 rabbit ani-human PeproTech, Rocky Hill, N J	42
hBD4	Abcam, San Francisco, California	41
TNF-α	TNF-α (code ab 6671, obtained from rabbit, 1:100 dilution, Abcam, Cambridge, UK	13
	559071 mAbG 1:10 mouse antihuman R&D Systems	14
IL-6	AbD Serotec, Oxford, England	41
IL-10	R&D Systems, Inc, Minneapolis, Minnesota	41
IL-12	IL-12p7024945 R&D Systems	32
IL-23	IL23p19 HLT2736 Biolegend	32
IL-17	clone AF-317-NA; R&D Systems, Wiesbaden Germany	30
	Polyclonal R& D Systems	32
IL-32	NBP-76684, Novus (Littleton, CO, U.S.A.)	4
IL-36	rabbit polyclonal anti-IL-36a (C-terminal; ab180909), rabbit polyclonal anti- IL-36b (C-terminal; ab180890) and mouse monoclonal anti-IL-36c (ab156783; all from Abcam, Cambridge, U.K.	6
	AF 1078,1099,2320,1275 RnD	5
Caspase1	clone 14F468; Imgenex/Novus Biologicals, Littleton, CO, U.S.A.)	30
NLRP3	clone Ab17267; Abcam, Cambridge, U.K.	30
MIF	MAB289 mAbG 1:100 mouse antihuman	14
S100A7	HL15-4 mAbG 1: 20,000 mouse antihuman Donated by Prof Schroders Labor Kiel germany	14
LL37/ Cathelicidin	Ab64892 pAbG 1:1000 rabbit antihuman Abcam	14
	Rabbit anti-human LL-37 [Abcam, Cambridge, UK	29
α-MSH	M0939 1:500 Rabbit Anithuman Sigma	14
	PROGEN Biotechnik GmbH, Heidelberg, Germany	41
Tryptase	AA1 1:800 Dako	12
	clone AA1; Dako	30

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staining cells were seen on the slopes of papillae of the epidermis[35]. hBD2 (human beta defensin) staining was decreased throughout the epidermis in two studies[13,41] whilst hBD3 staining was increased throughout the suprabasal epidermis[14,42], however only significantly in Hurley Stage 1 and 2 patients (p=0.045)[42]. hBD4 was decreased in suprabasal epidermis compared to healthy controls (p=0.001)[41]. Contradictory findings were seen in toll like receptor (TLR) 2 staining with an increase in the epidermis co-localizing with dendritic cells and macrophages in one study[40] but suppressed in a second study[41]. Levels of TLR3, TLR4, TLR7, TLR9, ICAM-1, TGF-Beta and IGF-1 were only assessed by one study and all were suppressed throughout the epidermis compared with controls[41]. RNAase7 was increased in expression compared to healthy controls ($p<0.05$)[42]. MMP2 was positively expressed in keratinocytes throughout the epidermis[13] and MMP8 in neutrophils within the epidermis[43]. TNF-α was highly expressed in macrophages and lymphocytes present in the epidermis, particular in the basal layers[13,14] and NLRP3, MIF, S100A7, LL37/Cathelicidin and α-MSH all positive in suprabasal keratinocytes[30,41]. IL-6 and IL-10 were reported as suppressed compared to healthy control skin[41], however, IL-36 subtypes were highly expressed in epidermal keratinocytes (more suprabasal than basal)[5,6] with IL-32 also positive in the stratum granulosum[4].

Dermis. CD1a, CD11c, CD206, CD207, CD209 and Factor XIIIa positive cells were identified in the dermis in three separate studies[12,30,40], however the degree of infiltration varied. Dermal infiltrates of CD3, CD4, and CD8 positive cells, continuous with the epidermal infiltrates were a consistent feature of lesional HS dermis and were increased over controls[30,33]. The distribution of these cells was most pronounced in the interfollicular dermis (ie. towards the papillary slopes) and perifollicularly (ie. peri-infundibularly)[30,33]. CD56, CD68 and CD138 positive cells were diffusely seen throughout the dermis[40]. CD19 and CD20 positive pseudolymphoid follicles have been noted in other studies[30,40]. Single keratinocytes have also been identified in the dermis which stain with pancytokeratin markers (AE1/AE3/PKC26)[36]. Inflammatory cells in the dermis co-localized with TNF-α[13,14], LL-37/cathelicidin[29], IL-12[32], IL-23[32], IL-17[30,32], IL-32[4], TLR2[40] and MMP8[43]. MMP2 co-localized with macrophages and fibroblasts[13]. IL-36 was not identified in the dermis[5,6].

Hair follicle. Cytokeratin staining of the follicular apparatus is consistent with normal K14, K16 and K17 staining. CD29 positive cells were identified in the infundibulum[35]. CD3, CD4, CD8, CD68, Factor XIIIa positive cells were seen within the outer root sheath (ORS) contiguous with dense peri-follicular inflammation in the adjacent dermis[30,33]. The presence of inflammatory cells co-localized with MMP2[13], TNF- α[13,14], and LL37/cathelcidicin[13,29]. hBD3[13,42] and MIF[13] also stained positive in the ORS. One conflicting study reported no change in TNF-α staining of the follicular unit[13].

Sinus tracts. Staining patterns differed between superficial keratinized sinus tracts and deeper, inflamed non-keratinized sinus tracts. Normal epidermal cytokeratin staining was seen in the keratinized superficial portion of sinus tracts including K1, K10, K14[36–38]. Ki67 was elevated and CD29 positive cells were also identified in sinus tracts[35]. Ki67 stained in both keratinized and non-keratinised portions of the sinus tract[38]. K19 staining was absent in keratinized portions of sinus tracts[37]. In deeper, inflamed, non-keratinized portions of the sinus tracts, K16, K17 and K19 were positive, with loss of K1, K10 and adhesions molecules including DG1 (desmoglein 1)and DSC1 (desmocollin 1)[37,38]. Apocrine gland nuclei stained weakly positive for estrogen receptor[39] and androgen receptor[39], and these results were reported as no different from control specimens[39]. Lysozyme staining of apocrine glands was seen in cases of vulval HS only[34].

Immunohistochemistry methods. The list of antibodies used for IHC staining is presented in Table 6. Consistent antibodies were used for CD1a; CD20 and tryptase staining, whilst different antibodies were used for other staining targets. Antibodies used were not described in two studies[34,36].

Assessment of Bias. The result of bias assessment using NIH criteria is presented in Table 7. All 22 articles clearly stated the research question of interest with well-defined study populations. The application of inclusion and exclusion criteria, or the calculation of sample size, or effect estimates were not described in any study. Exposures (ie. the presence of disease) were established and measured in all studies prior to the outcome measures (IHC staining) being assessed and the disease was established for such a time that a relationship between exposure and outcome would be identified if one existed. Different levels of exposure (severity of disease) was taken into account in only two studies[4,12] and was consistently measured using Hurley staging across all studies. No articles accounted for all possible confounding variables such as obesity, diabetes, family history or smoking status (Table 3).

Discussion

Quality of data and risk of bias

The overall quality of data in this systematic review was suboptimal with poor correction for potential confounding factors with only two of the 22 studies using objective measurement systems for IHC staining intensity[4,12]. The proportion of smokers was elevated (94%) compared to the rates of smoking in the HS population at large (70–89%)[45]. A number of studies (17/22) did not stratify results by treatment therefore there is a risk that staining intensity of pro-inflammatory mediators may be reduced due to concomitant treatment at the time of biopsy. The use of de-paraffinized tissue in retrospective studies[30,33,34] can lead to false negatives in IHC dependent upon the preparation method of the original sample and the de-paraffinization process[15]. Hence there are factors in the population studied in this review which may bring into question the reliability of staining quantification. However, the presence or absence of IHC staining, particularly when confirmed in multiple studies is still considered reliable despite the risks of bias.

Conflicting results

Conflicting results were identified in dermal CD1a staining[12,30,40], dermal CK19 staining[36–38], Epidermal TLR2 staining[40,41] and TNF alpha staining in the follicular infundibulum[13,14]. Regarding

Table 7. NIH Risk of Bias.

Study Reference	1. Was the research question or objective in this paper clearly stated?	2. Was the study population clearly specified and defined?	3. Was the participation rate of eligible persons at least 50%?	4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	5. Was a sample size justification, power description, or variance and effect estimates provided?	6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	10. Was the exposure(s) assessed more than once over time?	11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	12. Were the outcome assessors blinded to the exposure status of participants?	13. Was loss to follow-up after baseline 20% or less?	14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
Emelianov *et al.*[14]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Hessam *et al.*[6]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Thomi *et al.*[29]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Lima *et al.*[30]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Schlapbach *et al.*[32]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Thomi *et al.*[4]	Y	Y	N/A	N	N	Y	Y	Y	Y	N	Y	NR	N/A	N
Thomi *et al.*[5]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Wolk *et al.*[31]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Van der Zee *et al.*[12]	Y	Y	N/A	N	N	Y	Y	Y	Y	Y	Y	NR	N/A	N
Mozeika *et al.*[13]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Von Laffert *et al.*[33]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N

Study Reference	1. Was the research question or objective in this paper clearly stated?	2. Was the study population clearly specified and defined?	3. Was the participation rate of eligible persons at least 50%?	4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	5. Was a sample size justification, power description, or variance and effect estimates provided?	6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	10. Was the exposure(s) assessed more than once over time?	11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	12. Were the outcome assessors blinded to the exposure status of participants?	13. Was loss to follow-up after baseline 20% or less?	14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
Van der Zee *et al.*[11]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Heller *et al.*[34]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Gniadecki *et al.*[35]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Fismen *et al.*[36]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Kurokawa *et al.*[37]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Kurzen *et al.*[38]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Buiner *et al.*[39]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Hunger *et al.*[40]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Derno *et al.*[41]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Hofmann *et al.*[42]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Tsaousi *et al.*[43]	Y	Y	N/A	N	N	Y	Y	N	Y	N	Y	NR	N/A	N
Total	**22/22**	**22/22**	**N/A**	**0/22**	**0/22**	**22/22**	**22/22**	**2/22**	**22/22**	**1/22**	**22/22**	**NR**	**N/A**	**0/22**

Key: Y = Yes; N= No, NR= Not Reported N/A = Not Applicable

CD1a staining, two of the studies reported only a mild dermal infiltrate of CD1a positive cells[30,40], with a third study demonstrating a significant infiltration of these cells[12]. This third study clearly documented all treatment was withheld 3 weeks prior to the biopsies being taken[12], whereas there is no description in the other two articles regarding the discontinuation or ongoing use of treatments[30,40]. Therefore, with the possibility of partially treated disease, an artificial reduction in the number of dermal dendritic cells is a possibility as treatment for HS (such as adalimumab) has been demonstrated to effectively reduce the infiltration of dendritic cells *in vivo*[12]. Similarly, studies examining TNF-alpha staining also differed in their stratification of patient based upon active treatment[13,14]. Significant reductions in TNF alpha staining were seen in the study with no documentation of treatment cessation[14] when compared to the one study with clear documentation that all patients had treatment ceased prior to biopsy[13]. K19 staining was reported negative in all areas of the sinus tracts in one study[37], whereas two additional studies[36,38] described positive K19 staining in sinus tracts (one study non-specifically[36] and the second in the deep inflamed, non-keratinized epithelium of the tract[38]). The difference between these staining patterns may be explained by the presence of inflammation. Kurzen *et al.*[38] described the presence of K19 staining in non-keratinized epithelium of the deep sinus tracts only when associated with inflammation (Type 3 epithelia), staining was negative when no inflammation was present (Type 2 epithelia)[38]. Kurokawa *et al.* did not differentiate between inflamed and non-inflamed non-keratinized epithelium in their study[37], and noted that the lesser degree of inflammation seen histologically may explain their differing results in comparison to Kurzen's study[37].

Localization of production of inflammatory mediators

IHC staining, in particular co-staining with cellular markers and cytokines has enabled the localization of inflammatory mediators in order to ascertain the functional aspects of infiltrating inflammatory cells in HS, particularly highlighting the strong T_h17 polarity of inflammation in HS[3]. A schematic representation of the pathogenesis of HS based upon the findings of this review is presented in Figure 2. This highlights the interrelationship between inflammation and hyperkeratinization. Localization of TNF-α[13], IL-12[32], IL-23[32] and IL-32[4], TLR2[40], MMP2[13], MMP8[43] and LL-37/cathelicidin[14,29] production to infiltrating dermal macrophages and lymphocytes as well as localization of IL-36 subtypes[5], LL-37/cathelicidin[14,29], IL-1β[32] and IL-22[32] to keratinocytes illustrate the feed forward mechanisms similar to those seen in psoriasis[9] and atopic dermatitis[10] which likely contribute to persistent inflammation in HS. Rather than keratinocytes being innocent bystanders, these IHC findings demonstrate the central role keratinocytes play as producers of key inflammatory mediators as well as mediators of products (such as TGF-β and ICAM)[41] that may contribute to fibroblast dysregulation and hypertrophic scarring[46]. A remaining unanswered question includes the temporal relationship between keratinocyte hyperproliferation and the activation of inflammatory cells infiltrating the dermis and epidermis in HS.

Insights into pathophysiology of HS

The current pathophysiological paradigm of HS is one of follicular infundibular occlusion leading to follicle rupture and a resultant inflammatory cascade[1]. This paradigm was based on the pivotal work of Shelley and Cahn in 1955[47], whom demonstrated the induction of HS after application of belladonna impregnated tape to manually epilated axillae of 12 men. Only 3 of the 12 men developed the lesions described, and infection from the manual epilation procedure could not be excluded as a cause of the lesions, but this study enabled the paradigm to slowly shift away from one of apocrinitis, which had been in place since the original descriptions of the disease[7]. Detailed descriptions of infundibular hyperkeratosis (also termed poral occlusion) were made by Jemec *et al.*[7] and demonstrated the secondary involvement of apocrinitis in HS lesions. Jemec noted that poral occlusion was seen to occur alongside inflammation, but there was no suggestion of causation in one direction or another[7].

Although individual cases of epidermal hyperkeratosis in the absence of inflammation are noted[7,33], these cases are established or chronic lesions associated with significant fibrosis which is documented to be associated with reduce inflammatory infiltrate[7,33]. A consistent finding in all studies of this review is the co-localization of infundibular ORS keratinocyte hyperplasia with CD3, CD4, CD8 and CD68 positive inflammatory cells expressing TNF-α, IL-12, IL-23 and IL-32[4,13,32,40,43]. K19 is also documented as positive in the infundibulum suggesting keratinocyte hyperplasia[36–38]. However, it remains unclear whether keratinocyte hyperplasia induces the inflammatory cascade or if the inflammatory cascade induces the keratinocyte hyperplasia. The presence of inflammation in clinically normal, peri-lesional HS skin is well documented[4,30,33] implying the existence of a pre-clinical inflammation preceding symptoms of follicular occlusion. This is consistent with recent findings in acne pathogenesis that suggest that inflammation precede follicular hyperkeratosis and development of microcomedones[48] and is also pivotal in the ongoing development of nodulocystic acne and acne scars[49]. This pre-clinical inflammation is also consistent with the pathogenic paradigm in psoriasis and atopic dermatitis[9,10] with inflammation driving epidermal hyperkeratosis and alterations in keratinocyte maturation, consistent with the spongiform infundibulfolliculitis seen in established lesions of HS[50]. Our disparate findings in K19 staining in deep non-keratinized sinus tract epithelia with and without inflammation[37,38] also fit with this paradigm. In contrast, findings which would hold consistency with the current follicular occlusion paradigm would include infundibular occlusion preceding the development of inflammation, as well as alterations to desmosomal and hemidesmosomal proteins which would allow for rupture of the occluded follicles in order to drive the development of dermal inflammation and sinus tract formation. Although Danby *et al.*[51] reports reduced PAS positivity in the basement membrane zone at the sebo-follicular junction associated with inflammation in HS, it is likely that the reduced basement membrane integrity is secondary to inflammation and release of TGF-β and MMP2[52] (cytokines known to be altered in HS lesional skin and consistent with an abnormal wound healing response) rather than the follicular rupture being the primary driver of inflammation.

A more consistent hypothesis which accounts for the observed results of this review would be that of subclinical inflammation

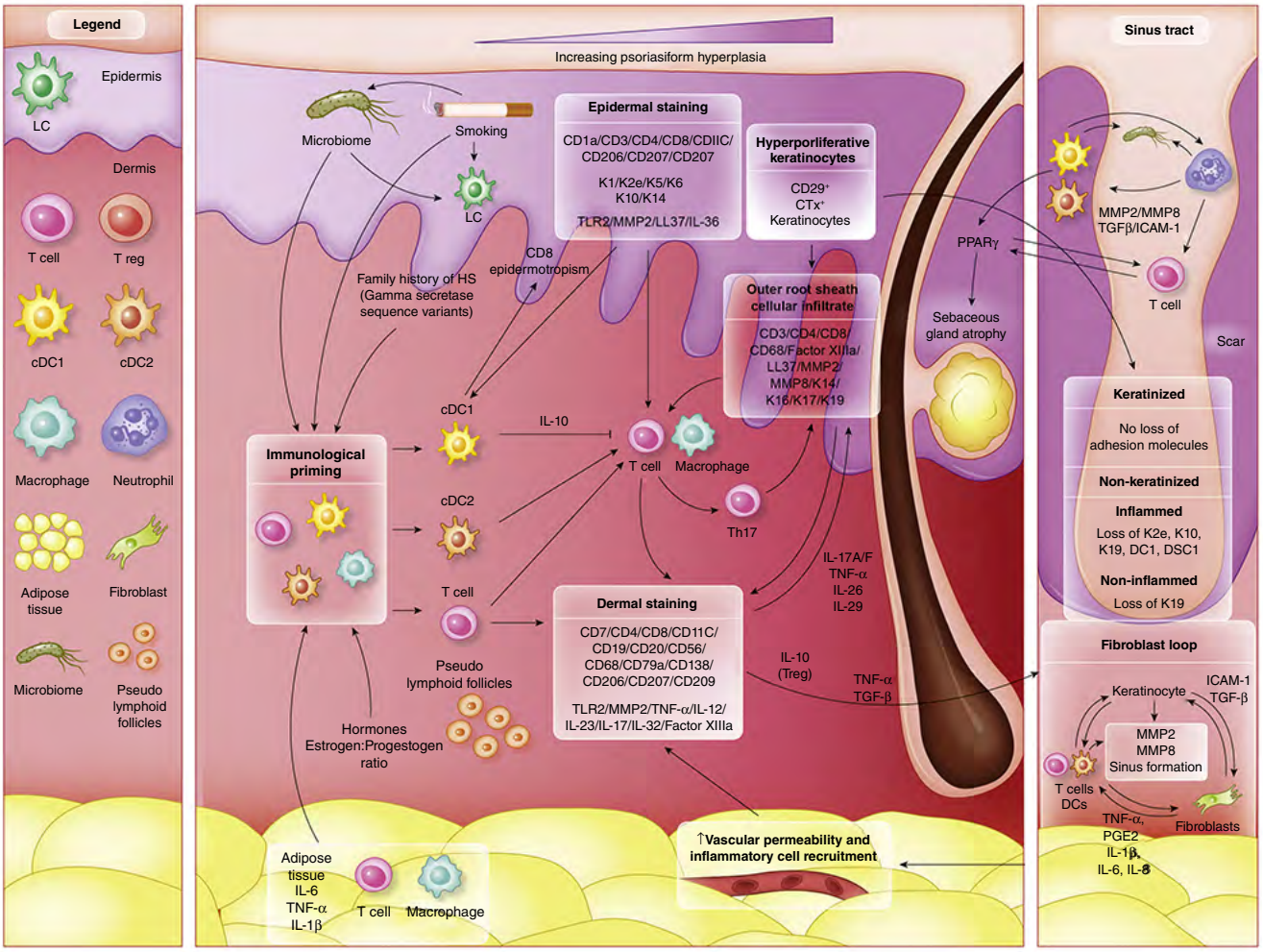

Figure 2. Schematic Representation of Immunohistochemical findings in hidradenitis suppurativa. Immunological 'priming' occurs due to the contribution of adipose tissue, genetic susceptibility, smoking-related inflammatory mediators and obesity related pro-inflammatory signals and the composition of the microbiome. Increased activity of cDC1, cDC2 and T cells lead to both keratinocyte hyperplasia via the actions of IL-12 and IL-23, as well as a T_h17 predominant immune response. Alterations of antimicrobial peptides (AMP's) also occur throughout the epidermis. IHC staining localize Langerhan cells and activated dendritic cells to the epidermis and the dermo-epidermal junction. A population of epidermotropic CD8 T cells are also present. IHC staining indicates a mixed inflammatory infiltrate in the dermis, with contributions from Dendritic cells, B cells, T cells and plasma cells. Within sinus tracts, adhesion molecules are preserved, but inflammation in associated with non-keratinised sinus tracts leads to a loss of K19. The development of scarring and sinus tracts is associated with MMP2, ICAM-1 and TGF-Beta, with possible augmentation of ICAM-1 and TGF-B signaling via specific components of the microbiome. TNF-a, PGE2 and CXCL2 then lead to additional feed forward mechanisms perpetuating the inflammatory cycle.

(due to a variety of triggers and immunological primers as illustrated in Figure 2) driving keratinocyte proliferation in the interfollicular epidermis and the follicular ORS, with follicular occlusion being a secondary phenomenon (mediated by TLR2 and IL-1α as documented in the development of comedones)[53]. The development of sinus tracts and hypertrophic scarring may also be mediated by the keratinocyte inflammatory response given the alterations in important wound healing mediators including TGF-β, ICAM-1 and comparisons by other authors of an altered wound healing response[8] in HS. This comparison would be appropriate given the high levels of dermal MMP2[13] and MMP8[43]; the loss of keratinocyte maturation markers (K2e, K10, K19)[36–38] adhesion molecules (DG1 and DCN2)[38] in the non keratinized inflamed epithelium of the deep dermis; suppressed levels of ICAM-1[41] (seen impaired wound healing[54]) and TGF-β[41] which leads to the dysregulation of TGF- β receptor ratio on fibroblasts which is linked with the development of hypertrophic scarring[46,54] seen in HS. These alterations to keratinocyte maturation are reminiscent of epithelial mesenchymal transition (EMT)[52] which may also explain the presence of free keratinocytes in the dermis in established lesions of HS[7,36]. Indeed, as ICAM-1 is up-regulated

by pro-inflammatory mediators[54], the low level of ICAM-1 noted appears paradoxical, however specific bacteria (including *Porphyromonas* species) which have been associated with HS[44,55] can suppress ICAM-1 production as an immune evasion strategy[56]. This implies that exogenous triggers (possibly including bacterial stimuli) can be a common cause for the initial inflammatory cascade as well as the development of tunneling and hypertrophic scarring in HS.

Conclusions

This systematic review of immunohistochemical staining of lesions in HS has highlighted the heterogeneity of studies and the methodological issues, which bring into question some of the results of IHC staining in HS lesions. The design of studies and variable reporting of potential confounding factors (such as ongoing or previous treatments) makes it impossible to compare staining intensity across studies. The results of existing studies suggest a florid inflammatory reaction comprising of T-lymphocytes, macrophages and dendritic cells with a strong Th-17 signature along with a keratinocyte mediated IL-36 inflammatory loop associated with keratinocyte hyperproliferation. The follicular occlusion paradigm as a primary driver of HS is unclear given the findings of this review and other histological and cytokine studies and inflammation as a primary driver of disease with secondary hyperkeratosis and occlusion is a plausible hypothesis.

Data availability

All data underlying the results are available as part of the article and no additional source data are required

Extended data

OSF: Extended data. Data collection sheet. https://doi.org/10.17605/OSF.IO/2JKPW[27]

Reporting guidelines

OSF: PRISMA Checklist for 'A systematic review and critical evaluation of immunohistochemical associations in hidradenitis suppurativa'. https://doi.org/10.17605/OSF.IO/2JKPW[27]

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References

1. Hoffman LK, Ghias MH, Lowes MA: **Pathophysiology of hidradenitis suppurativa.** *Semin Cutan Med Surg.* 2017; **36**(2): 47–54.
PubMed Abstract | Publisher Full Text

2. Melnik BC, John SM, Chen W, *et al.*: **T helper 17 cell/regulatory T-cell imbalance in hidradenitis suppurativa/acne inversa: the link to hair follicle dissection, obesity, smoking and autoimmune comorbidities.** *Br J Dermatol.* 2018; **179**(2): 260–272.
PubMed Abstract | Publisher Full Text

3. Moran B, Sweeney CM, Hughes R, *et al.*: **Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which is Corrected by Anti-TNF Therapy.** *J Invest Dermatol.* 2017; **137**(11): 2389–2395.
PubMed Abstract | Publisher Full Text

4. Thomi R, Yerly D, Yawalkar N, *et al.*: **Interleukin-32 is highly expressed in lesions of hidradenitis suppurativa.** *Br J Dermatol.* 2017; **177**(5): 1358–1366.
PubMed Abstract | Publisher Full Text

5. Thomi R, Kakeda M, Yawalkar N, *et al.*: **Increased expression of the interleukin-36 cytokines in lesions of hidradenitis suppurativa.** *J Eur Acad Dermatol Venereol.* 2017; **31**(12): 2091–2096.
PubMed Abstract | Publisher Full Text

6. Hessam S, Sand M, Gambichler T, *et al.*: **Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop.** *Br J Dermatol.* 2018; **178**(3): 761–767.
PubMed Abstract | Publisher Full Text

7. Jemec GB, Hansen U: **Histology of hidradenitis suppurativa.** *J AM Acad Dermatol.* 1996; **34**(6): 994–999.
PubMed Abstract | Publisher Full Text

8. Negus D, Ahn C, Huang W: **An update on the pathogenesis of hidradenitis suppurativa: implications for therapy.** *Expert Rev Clin Immunol.* 2018; **14**(4): 275–283.
PubMed Abstract | Publisher Full Text

9. Hawkes JE, Chan TC, Krueger JG: **Psoriasis pathogenesis and the development of novel targeted immune therapies.** *J Allergy Clin Immunol.* 2017; **140**(3): 645–653.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Brunner PM, Guttman-Yassky E, Leung DY: **The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies.** *J Allergy Clin Immunol.* 2017; **139**(4S): S65–S76.
PubMed Abstract | Publisher Full Text | Free Full Text

11. van der Zee HH, de Ruiter L, van den Broecke DG, *et al.*: **Elevated levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-α and IL-1β.** *Br J Dermatol.* 2011; **164**(6): 1292–1298.
PubMed Abstract | Publisher Full Text

12. van der Zee HH, Laman JD, de Ruiter L, *et al.*: **Adalimumab (antitumour necrosis factor-α) treatment of hidradenitis suppurativa ameliorates skin inflammation: an *in situ* and *ex vivo* study.** *Br J Dermatol.* 2012; **166**(2): 298–305.
PubMed Abstract | Publisher Full Text

13. Mozeika E, Pilmane M, Nürnberg BM, *et al.*: **Tumour necrosis factor-alpha and matrix metalloproteinase-2 are expressed strongly in hidradenitis suppurativa.** *Acta Derm Venereol.* 2013; **93**(3): 301–304.
PubMed Abstract | Publisher Full Text

14. Emelianov VU, Bechara FG, Glasera R, *et al.*: **Immunohistological pointers to a possible role for excessive cathelicidin (LL-37) expression by apocrine sweat glands in the pathogenesis of hidradenitis suppurativa/acne inversa.** *Br J Dermatol.* 2012; **166**(5): 1023–1034.
PubMed Abstract | Publisher Full Text

15. Wester K, Wahlund E, Sundström C, *et al.*: **Paraffin section storage and immunohistochemistry. Effects of time, temperature, fixation, and retrieval protocol with emphasis on p53 protein and MIB1 antigen.** *Appl Immunohistochem Mol Morphol.* 2000; **8**(1): 61–70.
PubMed Abstract

16. Ergun T: **Hidradenitis suppurativa and the metabolic syndrome.** *Clin Dermatol.* 2018; **36**(1): 41–47.
PubMed Abstract | Publisher Full Text

17. Garg A, Birabaharan M, Strunk A: **Prevalence of type 2 diabetes mellitus among patients with hidradenitis suppurativa in the United States.** *J Am Acad Dermatol.* 2018; **79**(1): 71–76.
PubMed Abstract | Publisher Full Text

18. Lukach AJ, Saul MI, Ferris LK, *et al.*: **Risk Factors for Hidradenitis Suppurativa in Patients with Inflammatory Bowel Disease.** *Dig Dis Sci.* 2018; **63**(3): 755–760.
PubMed Abstract | Publisher Full Text

19. Garg A, Papagermanos V, MIdura M, *et al.*: **Incidence of hidradenitis suppurativa among tobacco smokers: a population-based retrospective analysis in the U.S.A.** *Br J Dermatol.* 2018; **178**(3): 709–714.
PubMed Abstract | Publisher Full Text

20. Sgambato JA, Jones BA, Caraway JW, *et al.*: **Inflammatory profile analysis reveals differences in cytokine expression between smokers, moist snuff users, and dual users compared to non-tobacco consumers.** *Cytoine.* 2018; **107**: 43–51.
PubMed Abstract | Publisher Full Text

21. Spoto B, Di Betta E, Mattace-Raso F, *et al.*: **Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity.** *Nutr Metab Cardiovasc Dis.* 2014; **24**(10): 1137–43.
PubMed Abstract | Publisher Full Text

22. Rehman K, Akash MS: **Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked?** *J Biomed Sci.* 2016; **23**(1): 87.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Zhou X, Fragala MS, McElhaney JE, *et al.*: **Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research.** *Curr Opin Clin Nutr Metab Care.* 2010; **13**(5): 541–547.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Frew J, Hawkes J, Krueger J: **A Systematic Review of Immunohistochemical studies in Hidradenitis Suppurativa.** *PROSPERO.* 2018; CRD42018104763. Accessed 27 August 2018.
Reference Source

25. Moher D, Liberati A, Tetzlaff J, *et al.*: **Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.** *PLoS Med.* 2009; **6**(7): e1000097.
PubMed Abstract | Publisher Full Text | Free Full Text

26. von Elm E, Altman DG, Egger M, *et al.*: **The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies.** *J Clin Epidemiol.* 2008; **61**(4): 344–9.
PubMed Abstract | Publisher Full Text

27. Frew J: **A Systematic Review and Critical Evaluation of Immunohistochemical Associations in Hidradenitis Suppurativa.** 2018.
http://www.doi.org/10.17605/OSF.IO/2JKPW

28. NIH NIoH: **Quality Assessment Tool for Observational Cohort and Cross Sectional Studies.** 2018. Accessed 30 July 2018.
Reference Source

29. Thomi R, Schlapbach C, Yawalkar N, *et al.*: **Elevated levels of the antimicrobial peptide LL-37 in hidradenitis suppurativa are associated with a Th1/Th17 immune response.** *Exp Dermatol.* 2018; **27**(2): 172–177.
PubMed Abstract | Publisher Full Text

30. Lima AL, Karl I, Giner T, *et al.*: **Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa.** *Br J Dermatol.* 2016; **174**(3): 514–521.
PubMed Abstract | Publisher Full Text

31. Wolk K, Wenzel J, Tsaousi A, *et al.*: **Lipocalin-2 is expressed by activated granulocytes and keratinocytes in affected skin and reflects disease activity in acne inversa/hidradenitis suppurativa.** *British J Dermatol.* 2017; **177**(5): 1385–1393.
PubMed Abstract | Publisher Full Text

32. Schlapbach C, Hanni T, Yawalkar N, *et al.*: **Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa.** *J Am Acad Dermatol.* 2011; **65**(4): 790–8.
PubMed Abstract | Publisher Full Text

33. von Laffert M, Stadie V, Wohlrab J, *et al.*: **Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae.** *Br J Dermatol.* 2011; **164**(2): 367–371.
PubMed Abstract | Publisher Full Text

34. Heller DS, Haefner HK, Hameed M, *et al.*: **Vulvar hidradenitis Suppurativa. Immunohistochemical evaluation of apocrine and eccrine involvement.** *J Reprod Med.* 2002; **47**(9): 695–700.
PubMed Abstract

35. Gniadecki R, Jemec GB: **Lipid raft-enriched stem cell-like keratinocytes in the epidermis, hair follicles and sinus tracts in hidradenitis suppurativa.** *Exp Dermatol.* 2004; **13**(6): 361–363.
PubMed Abstract | Publisher Full Text

36. Fismen S, Ingvarsson G, Moseng D, *et al.*: **A clinical-pathological review of hidradenitis suppurativa: using immunohistochemistry one disease becomes two.** *APMIS.* 2012; **120**(6): 433–440.
PubMed Abstract | Publisher Full Text

37. Kurokawa I, Nishijima S, Kusomoto K, *et al.*: **Immunohistochemical study of cytokeratins in hidradenitis suppurativa (acne inversa).** *J Int Med Res.* 2002; **30**(2): 131–136.
PubMed Abstract | Publisher Full Text

38. Kurzen H, Jung EG, Hartschuh W, *et al.*: **Forms of epithelial differentiation of draining sinus in acne inversa (hidradenitis suppurativa).** *Br J Dermatol.* 1999; **141**(2): 231–239.
PubMed Abstract | Publisher Full Text

39. Buiner MG, Wobbes T, Klinkenbilj JH, *et al.*: **Immunohistochemical analysis of steroid hormone receptors in hidradenitis suppurativa.** *Am J Dermatopathol.* 2015; **37**(2): 129–132.
PubMed Abstract | Publisher Full Text

40. Hunger RE, Surovy AM, Hassan AS, *et al.*: **Toll-like receptor 2 is highly expressed in lesions of acne inversa and colocalizes with C-type lectin receptor.** *Br J Dermatol.* 2008; **158**(4): 691–697.
PubMed Abstract | Publisher Full Text

41. Dreno B, Khammari A, Brocard A, *et al.*: **Hidradenitis suppurativa: the role of deficient cutaneous innate immunity.** *Arch Dermatol.* 2012; **148**(2): 182–186.
PubMed Abstract | Publisher Full Text

42. Hofmann SC, Saborowski V, Lange S, *et al.*: **Expression of innate defense antimicrobial peptides in hidradenitis suppurativa.** *J Am Acad Dermatol.* 2012; **66**(6): 966–974.
PubMed Abstract | Publisher Full Text

43. Tsaousi A, Witte E, Witte K, *et al.*: **MMP8 Is Increased in Lesions and Blood of Acne Inversa Patients: A Potential Link to Skin Destruction and Metabolic Alterations.** *Mediators Inflamm.* 2016; **2016**: 4097574.
PubMed Abstract | Publisher Full Text | Free Full Text

44. Ring HC, Thorsen J, SAunte DM, *et al.*: **The Follicular Skin Microbiome in Patients With Hidradenitis Suppurativa and Healthy Controls.** *JAMA Dermatol.* 2017; **153**(9): 897–905.
PubMed Abstract | Publisher Full Text | Free Full Text

45. Micheletti RG: **Hidradenitis suppurativa: current views on epidemiology, pathogenesis, and pathophysiology.** *Semin Cutan Med Surg.* 2014; **33**(3 Suppl): S48–S50.
PubMed Abstract | Publisher Full Text

46. Zhu Z, Ding J, Tredget EE: **The molecular basis of hypertrophic scars.** *Burns Trauma.* 2016; **4**: 2.
PubMed Abstract | Publisher Full Text | Free Full Text

47. Shelley WB, Cahn MM: **The pathogenesis of hidradenitis suppurativa in man; experimental and histologic observations.** *AMA Arch Derm.* 1955; **72**(6): 562–565.
PubMed Abstract | Publisher Full Text

48. Del Rosso JQ, Kircik LH: **The sequence of inflammation, relevant biomarkers, and the pathogenesis of acne vulgaris: what does recent research show and what does it mean to the clinician?** *J Drugs Dermatol.* 2013; **12**(8 Suppl): s109–115.
PubMed Abstract

49. Holland DB, Jeremy AH: **The role of inflammation in the pathogenesis of acne and acne scarring.** *Semin Cutan Med Surg.* 2005; **24**(2): 79–83.
PubMed Abstract | Publisher Full Text

50. Boer J, Weltvreden EF: **Hidradenitis suppurativa or acne inversa. A clinicopathological study of early lesions.** *Br J Dermatol.* 1996; **135**(5): 721–5.
PubMed Abstract | Publisher Full Text

51. Danby FW, Jemec GB, Marsch WCh, *et al.*: **Preliminary findings suggest hidradenitis suppurativa may be due to defective follicular support.** *Br J Dermatol.* 2013; **168**(5): 1034–1039.
PubMed Abstract | Publisher Full Text

52. Kalluri R, Weinberg RA: **The basics of epithelial-mesenchymal transition.** *J Clin Invest.* 2009; **119**(6): 1420–1428.
PubMed Abstract | Publisher Full Text | Free Full Text

53. Selway JL, Kurczab T, Kealey T, *et al.*: **Toll-like receptor 2 activation and comedogenesis: implications for the pathogenesis of acne.** *BMC Dermatol.* 2013; **13**: 10.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Koivisto L, Heino J, Hakkinne L, *et al.*: **Integrins in Wound Healing.** *Adv Wounds Care (New Rochelle).* 2014; **3**(12): 762–783.
PubMed Abstract | Publisher Full Text | Free Full Text

55. Guen-Revillet H, Jais JP, Ungeheuer MN, *et al.*: **The Microbiological Landscape of Anaerobic Infections in Hidradenitis Suppurativa: A Prospective Metagenomic Study.** *Clin Infec Dis.* 2017; **65**(2): 282–291.
PubMed Abstract | Publisher Full Text

56. Huang GT, Kim D, Lee JK, *et al.*: **Interleukin-8 and intercellular adhesion molecule 1 regulation in oral epithelial cells by selected periodontal bacteria: multiple effects of Porphyromonas gingivalis via antagonistic mechanisms.** *Infect Immun.* 2001; **69**(3): 1364–1372.
PubMed Abstract | Publisher Full Text | Free Full Text

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Version 2

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✔ **Martin M. Okun**
Fort HealthCare, Fort Atkinson, WI, USA

My concerns have been satisfactorily addressed with these changes. I support indexing.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 19 March 2019

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? **Martin M. Okun**
Fort HealthCare, Fort Atkinson, WI, USA

This is a thorough, thoughtful, and valuable contribution to the scientific literature on pathogenesis of hidradenitis suppurativa.

The principal advances of the systematic review are:

- cogently advances a reasonable hypothesis to explain decreased levels of inflammatory marker density in some studies as due to the lack of treatment interruption;

- links the presence of certain hyperproliferative keratin markers (K19) to the presence of concomitant inflammation
- collates evidence from multiple studies demonstrating the presence of keratinocyte-derived pro-inflammatory biomarkers, reinforcing the concept that keratinocytes are actively contributing to the inflammatory milieu

The authors advance the hypothesis that follicular occlusion is secondary to inflammation, based on:

- absence of evidence of follicular occlusion without inflammation (though absence of evidence is not equivalent to the evidence of absence)
- presence of inflammation in clinically normal perilesional skin (though inflammation could be spill-over from adjacent inflamed skin)
- analogies with other inflammatory skin diseases
- presence of K19 staining only in inflamed sinus tracts (though the relevance of this observation for the pathogenesis of HS is uncertain)

In short, this hypothesis is plausible but the conclusion that "primary follicular occlusion as a pathogenic paradigm and the principal driver of HS is not consistent with the findings of this review" seems too sweeping a statement based on the information provided. The authors should consider altering this conclusion in line with the limitations of available data.

As a minor issue, there is an unnecessary repetition in the second sentence of the Immunohistochemistry results, epidermis section: "Increased K6, K16 and K17 staining were increased..."

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 February 2019

https://doi.org/10.5256/f1000research.18880.r42315

Gregor B. E. Jemec
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The authors provide a systematic review of immunohistochemical studies of hidradenitis suppurativa (HS).
The clearly stated objectives are:

1. To collate and describe all published reports of immunohistochemical studies in HS.
2. To critically evaluate the sampling, laboratory and analysis techniques used in each study to determine if comparisons can be made across studies.

The review was registered with PROSPERO and conducted in line with the PRISMA. The STROBE statement was used to assess the observational studies included in the study. A PRISMA flow chart and a search strategy are provided accordingly.

The authors adequately discuss the confounding factors and risk of bias, which both are significant weaknesses identified in the literature by this manuscript based on limited studies.

Only 22 articles were identified describing results from 494 HS patients (average 22 pts/study) and only 168 controls. Furthermore, 87 unique immunohistochemical targets were identified adding to the scarcity of hard data. It is therefore less surprising that conflicting data were found. The authors are however able to provide a realistic analysis of the data taking these limitations into account, and, in addition, provide coherent analyses and a testable paradigm for the pathomechanisms of HS.

The paper provides an excellent overview of the limited number of explorative immunohistochemical studies of HS, and thus provides an important stepping-stone to further studies.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

4.1.3: Publication 1-3

Frew JW, Hawkes JE, Krueger JG "Topical, Systemic and Biologic Therapies in Hidradenitis Suppurativa: Pathogenic Insights Through Examination of Therapeutic Mechanisms" (2018) Therapeutic Advances in Chronic Disease 2019; 10: 2040622319830646.

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Topical, systemic and biologic therapies in hidradenitis suppurativa: pathogenic insights by examining therapeutic mechanisms

John W. Frew, Jason E. Hawkes and James G. Krueger

***Abstract*:** Hidradenitis suppurativa (HS) is a chronic inflammatory disease of the skin, manifesting in chronic, recurrent painful pustules, nodules, boils and purulent draining abscesses. Our current understanding of the pathogenesis of the disease is incomplete. This review aims to identify available treatment options in HS and discuss the pharmacological mechanisms through which such agents function. Identifying common pathways may inform our understanding of the pathogenesis of HS as well as identify future therapeutic targets. The pharmacological mechanisms implicated in topical therapies, antibiotic, hormonal, systemic immunomodulatory and biologic therapies for HS are discussed. Significant differences exist between agents and implicated pathways in therapy for mild and severe disease. This is an expression of the possible dichotomy in inflammatory pathways (and treatment responses) in HS. Studies involving monoclonal antibodies provide the greatest insight into what these specific mechanisms may be. Their variable levels of clinical efficacy compared with placebo bolsters the suggestion that differential inflammatory pathways may be involved in different presentations and severity of disease. Nuclear factor kappa B (NF-κB), tumor necrosis factor (TNF)-α and other innate immune mechanisms are strongly represented in treatments which are effective in mild to moderate disease in the absence of scarring or draining fistulae, however complex feed-forward mechanisms in severe disease respond to interleukin (IL)-1 inhibition but are less likely to respond to innate immune inhibition (through NF-κB or TNF-α) alone. It is unclear whether IL-17 inhibition will parallel TNF-α or IL-1 inhibition in effect, however it is plausible that small molecule targets (Janus kinase1 and phosphodiesterase 4) may provide effective new strategies for treatment of HS.

Keywords: biologics, cytokines, hidradenitis suppurativa, interleukin-17, inflammation, tetracycline, tumor necrosis factor-α

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Background

Hidradenitis suppurativa (HS) is a chronic inflammatory disease of the skin, manifesting in chronic, recurrent, painful pustules, nodules, boils and purulent draining abscesses.[1] It commonly affects the flexural areas of the axillae, sub-mammary folds, inguinal and gluteal regions. It has an estimated prevalence of 1–4%[2] similar to other common dermatoses such as atopic dermatitis and psoriasis. Representative clinical manifestations of HS are presented in Figure 1.

HS affects women three times more than men, with those of lower socioeconomic status disproportionately affected.[1,2] Up to one-third of cases of HS are associated with pathogenic sequence variants identified in gamma secretase subunits as well as the inflammatory pathways associated with

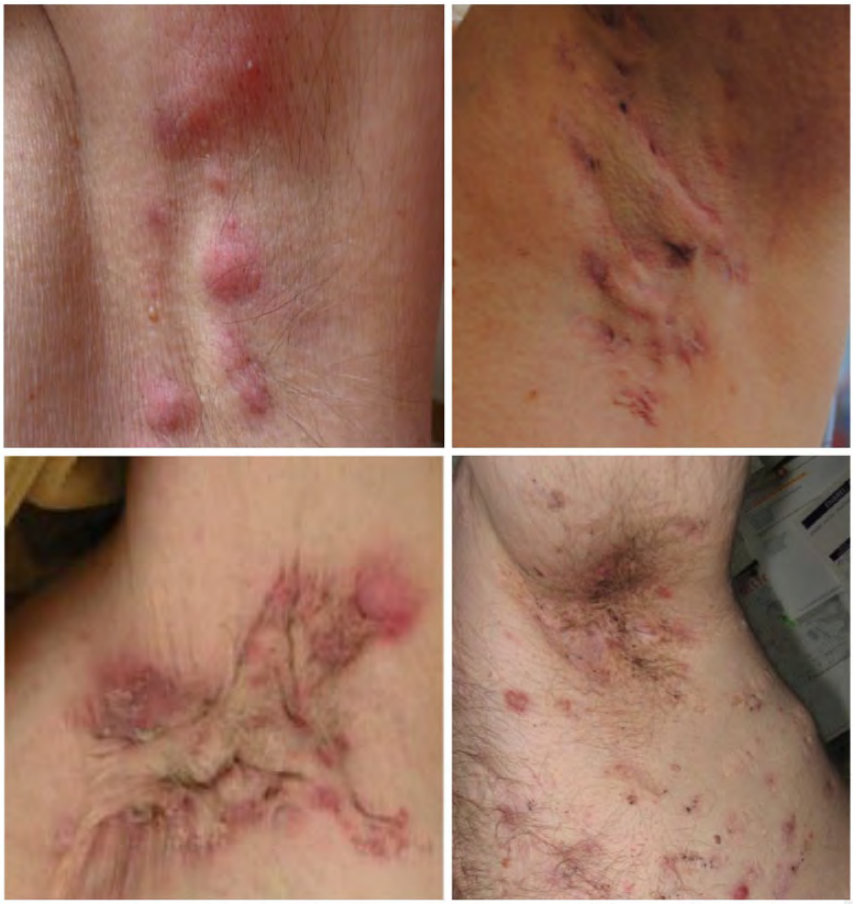

Figure 1. Clinical manifestations of hidradenitis suppurativa (HS) demonstrating inflammatory nodules in Hurley Stage 1 disease (top left), sinus tracts and inflammatory nodules separated by largely normal skin (Hurley Stage 2) (top right); widespread scarring inflammation and interconnected sinus tracts (Stage 3), (Bottom left) and follicular scarring and double ended comedones in PASH (Pyoderma Gangrenosum, Acne Congolobata and Suppurative Hidradenitis) Syndrome (Bottom right).

autoinflammatory diseases.[3] Cigarette smoking, obesity, diabetes, inflammatory bowel disease and arthritis are all associated with HS.[1,2] It has a significant impact upon quality of life, psychosexual function, and represents a notable financial and clinical burden on healthcare systems.[4] Potential complications include secondary infection, hypertrophic scarring, development of squamous cell carcinoma, chronic pain, psychological and psychosexual complications.[1] It is well documented that a lack of awareness and effective treatments contribute to diagnostic delay in HS, with an average of 7 years between onset and diagnosis.[5] For many years, HS has been considered an orphan disease with very few effective treatments available. Diagnostic criteria for the disease are presented in Table 1.

Over the past decade, a flourish of basic and clinical research has led to an increase of awareness of HS, and the treatment options for patients. Recent United States Food and Drug Administration (US FDA) approval of adalimumab for HS[7] has led to an increase in treatment options for disease control. However, our understanding of the disease pathogenesis remains incomplete.[8] Multiple ongoing trials are testing existing monoclonal antibodies with the hope of identifying effective therapies and providing insight into the pathogenic mechanisms underlying HS.[9,10] Whilst 'trial by therapy' does not systematically analyze the cellular mechanism underlying HS, alterations in the disease state can give inferential data regarding the role of specific inflammatory pathways in HS. Such data have challenged the existing pathogenic paradigm in HS. Currently, HS is considered a disease of follicular occlusion, which leads to the rupture and dermal seeding of bacteria and cellular debris[1,12]

Table 1. Diagnostic criteria for HS as defined by the European S1 guideline for the treatment of HS/acne inversa.[6] The presence of primary positive diagnostic criteria (either history or signs) are required and the presence of secondary positive diagnostic criteria are supportive of the diagnosis of HS.

Primary positive diagnostic criteria:
• History: More than two recurrent, painful or suppurating lesions over a period of 6 months • Signs: Involvement of axilla, genitofemoral area, perineum, gluteal area and inframammary area of women. Presence of nodules, sinus tracts, abscesses, scarring.
Secondary positive diagnostic criteria:
• History: A family history of HS • Microbiology: A negative swab or presence of normal skin microbiota may be indicative of HS.

HS, hidradenitis suppurativa.

leading to the influx of inflammatory cells and mediators. The response of HS to anti-inflammatory therapies has suggested that follicular occlusion may be a secondary phenomenon due to an aberrant inflammatory response to the cutaneous microbiome.[8] A schematic illustration of inflammatory pathways in HS is presented in Figure 2.

The purpose of this review is to provide a comprehensive overview of available treatment options in HS and to discuss the pharmacological mechanisms through which these agents function. This review aims to include a variety of therapies used in HS, not only those used by dermatologists, or those supported by clinical evidence, due to the fact that a paucity of high-level clinical evidence exists to guide treatment decisions in HS. Evaluations of the level of evidence for HS therapies are available from other high-quality publications.[6,13] Through this broad review of therapies, we aim to identify common mechanistic pathways among these agents, allowing us to present an updated, testable model of HS and its immunopathogenesis and identify potential future therapeutic targets.

Topical therapies in HS

Topical therapy is seen as the mainstay of treatment in mild to moderate HS,[6] although the level of evidence for its use is low[13] (Table 2). A variety of topical medicaments with biocidal as well as anti-inflammatory mechanisms are used, with varying degrees of benefit dependent upon disease severity. It is likely that individual patient factors (including the relative contribution of the microbiome to inflammation in HS) may alter the response to topical therapy. Observed benefits include reducing the frequency of secondary infection[14] but little evidence exists as to their efficacy in reducing flares of nodules, pustules, abscesses or preventing progression to more severe disease.

Chlorhexidine

Chlorhexidine is a biguanide broad spectrum biocide, the efficacy of which is highly pH dependent, with a maximum effect occurring within 20 s.[16] Even in high concentrations, decreased biocidal activity was seen in the presence of biofilms [including *Pseudomonas* sp., *methicillin-resistant Staphylococcus aureus* (MRSA) and *Escherichia coli*].[17] Given the established association of biofilms[18] and Gram-negative bacteria (*Porphyromonas* and *Peptidophillus* sp.)[19] with disease activity in HS, chlorhexidine may reduce the stimulation of the immune system by resident bacteria, but not in the presence of biofilms. Clinical evidence for the use of chlorhexidine is low, and benefit is derived only from reducing the incidence of bacterial resistance compared with oral antibacterial therapy.[14]

Topical povidone iodine

Povidone iodine is reported in the treatment of HS.[20] It demonstrates rapid bactericidal, tuberculocidal and viricidal effects through the release of free iodine radicals which attack free amino acids (methionine and cysteine).[16] This results in destabilization of membrane fatty acids through reactions with unsaturated carbon bonds. Free oxidation of other vital pathogen structures (phospholipid, DNA/RNA/membrane-bound proteins)

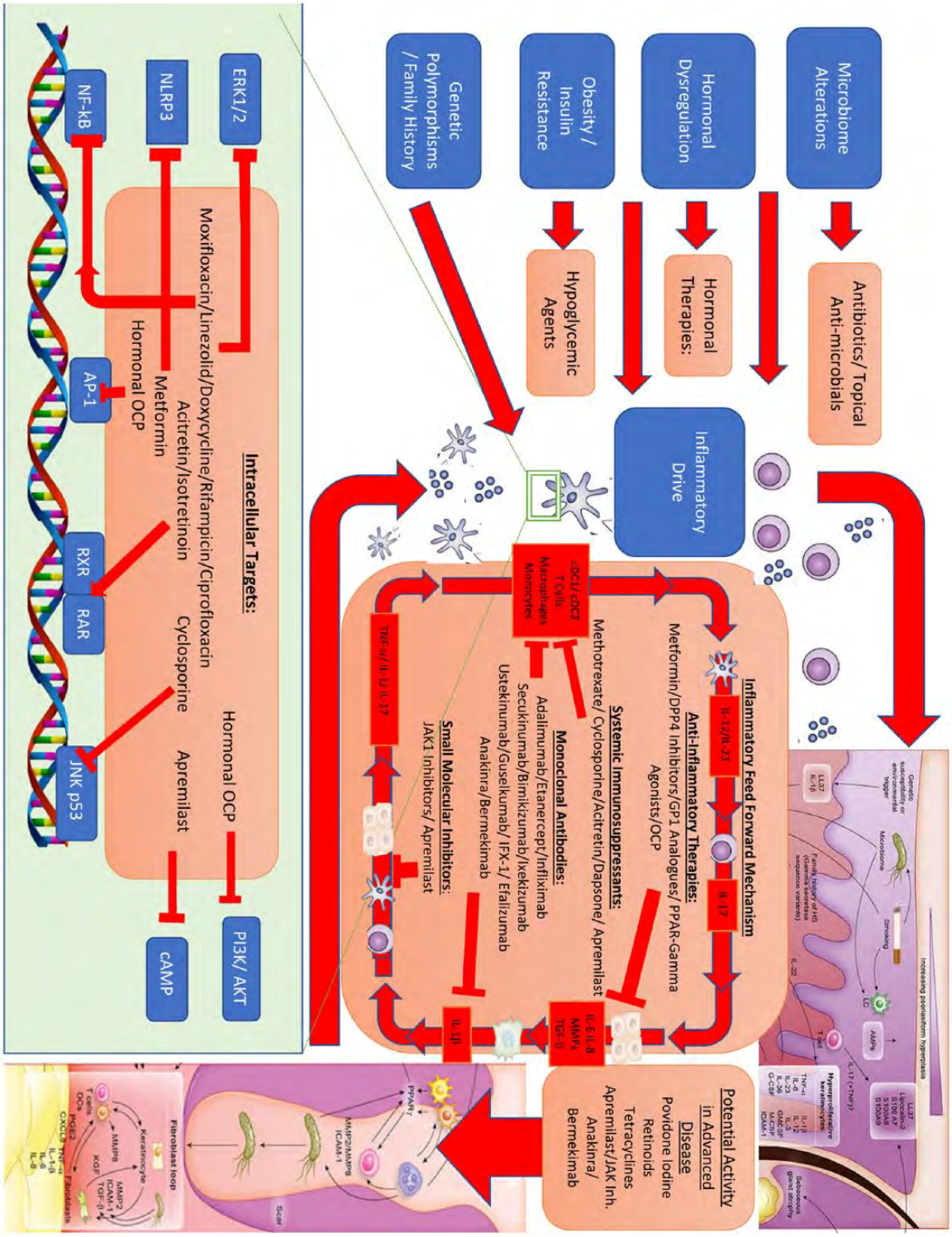

Figure 2. Pathogenesis of hidradenitis suppurativa and site of action of reported treatment modalities.

also occurs.[21] Iodine also has multiple anti-inflammatory properties which function through the inhibition of matrix metalloproteinase (MMP) production, reduction in plasmin activity, and inhibition of tumor necrosis factor alpha (TNF-α).[21] The role of MMP and TNF-α in HS[8] may

Table 2. Topical therapies reported in HS and descriptions of antibacterial, keratolytic and anti-inflammatory effects. The associated quality of evidence supporting the use of topical therapies is reported in the far-right hand side column.

Topical therapy	Mechanism(s) of action			Quality of evidence[15]
	Antibacterial effect	Keratolytic effect	Anti-inflammatory effect	
Chlorhexidine	Cell wall binding K+ efflux Poor effect on biofilm	Nil	Nil	C
Povidone iodine	Free radical oxidation of DNA/RNA/membrane proteins Some biofilm activity	Nil	Inhibition of MMP production, TNF-α	C
Pyrithione zinc	Bacteriostatic and antifungal: disruption of ATP and protein synthesis. Modulates cellular copper influx. Effect against yeast biofilms	Nil evidence	Only in the presence of intracellular zinc ions. Can increase TNF-α and HSP-70 in keratinocytes at high concentrations	C
Hydrogen peroxide	Free Radical Oxidation of DNA/RNA/Membrane proteins Some biofilm activity	Possible direct oxidation: nil evidence	Decrease ubiquination in NFκB pathway but higher concentrations display deleterious effects	C
Sodium hypochlorite	Free radical oxidation of DNA/RNA/membrane proteins High biofilm activity	Possible direct oxidation: nil evidence	Decreases NF-κB signaling	C
Triclosan	Disruption of bacterial wall synthesis	Nil	Downregulates TLR signaling, IL-6, IL-1ß expression	C
Clindamycin	Bacteriostatic effect 50S ribosomal binding	Nil	NF-κB and AP-1 gene expression, regulation of macrophage function., reduction in expression of virulence factors	C
Azelaic Acid	Bacteriostatic and antifungal	Nil	Modulation of PPAR-γ function, reduction in IL-6, IL-1ß TNF activity	C
Retinoids	Nil	Indirect effect through AP-1 and NF-κB modulation	NF-κB and AP-1 modulation, TLR2, MMP1 and MMP9 downregulation	C
Resorcinol	Membrane damage and K+ efflux	Minor at reported concentrations	Reported but mechanisms not described	C

AP-1, activator protein 1; ATP, adenosine triphosphate; HS, hidradenitis suppurativa; IL, interleukin; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; PPAR, peroxisome proliferator-activated receptor; TNF, tumor necrosis factor; TLR, Toll-like receptor.

partially explain the effect. Surprisingly little published evidence surrounding the use of oral Saturated Solution of Potassium Iodide (SSKI) for HS and this would be an area to explore further in controlled clinical trials.

Topical pyrithione zinc

Pyrithione zinc is a coordination complex of zinc present in a number of anti-dandruff products. It has fungistatic and bacteriostatic properties which function *via* the disruption of adenosine triphosphate (ATP) levels and protein synthesis.[22] Pyrithione zinc may also have some anti-inflammatory properties. Intracellular zinc can modulate the lipopolysaccharide (LPS)-stimulated maturation of dendritic cells *via* Toll-like receptors (TLRs);[23] however, the action of pyrithione zinc is dependent upon adequate intracellular zinc and excessive concentrations can exert a pro-inflammatory effect.[24] The clinical significance of the anti-inflammatory mechanisms of zinc is unclear as there is no evidence correlating the intake of dietary zinc to serum inflammatory markers in epidemiological studies.[25] Other concerns include the pro-estrogenic action of zinc pyrithione (ER bioactivity = 0.237) which is comparable to the clinically relevant exposure to butyl parabens (ER bioactivity = 0.251).[26]

Hydrogen peroxide

Hydrogen peroxide is a widely available biocide with nonspecific activity against viruses, bacteria, yeasts and spores.[16] It has greater activity against Gram-positive organisms; however, catalase positive organisms are more resistant at lower concentrations.[16] The risk of air emboli has been reported when hydrogen peroxide is used in highly vascular enclosed cavities in hypovolemic patients. However, this complication has not been reported in HS patients. Hydrogen peroxide is 266-times less effective against biofilms than free bacteria,[27] however efficacy can be increased with short contact times and novel irrigation methods in HS.[28] Its use is reported in HS[28] but no formal clinical studies have been undertaken. Alcohol-based formulations require longer exposure times to achieve the same bactericidal activity.[16] Anti-inflammatory effects have been described *in vitro* through decreased ubiquitination in the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway leading to a reduction in TLR4 signaling after LPS stimulation at low concentrations.[29,30] However, increased apoptosis and oxidative stress were observed at higher concentrations.[31]

Bleach baths (sodium hypochlorite solution)

Dilute bleach baths (sodium hypochlorite) are a well-established antimicrobial and anti-inflammatory treatment for atopic dermatitis,[31] and its use has been extended to include HS. Dilute sodium hypochlorite is bactericidal *via* direct oxidative reactions with bacterial proteins as well as inhibition of bacterial growth with as little as 5 min of exposure.[16] DNA synthesis is much more sensitive to sodium hypochlorite than protein synthesis at low concentrations[32] and this is the likely mechanism in HS. It demonstrates some activity against biofilms, but has incomplete bactericidal activity even at high concentrations.[33,34] No clinical studies have estimated the efficacy of bleach baths in HS; however, it may have anti-inflammatory activity mediated *via* modulation of NF-κB signaling[32,35]

Triclosan

Triclosan is a halogenated bisphenol derivative with broad spectrum bactericidal activity (excluding *Pseudomonas* sp.).[36,37] It achieves this through disruption of bacterial cell wall synthesis.[16,37] The efficacy of triclosan is highly dependent upon the formulation used, and increased potency is known to occur with ethylenediaminetetraacetic acid (EDTA).[16] Triclosan is recommended in the HS literature for Hurley stage 1 and 2 disease.[38] Triclosan exerts an anti-inflammatory effect independent of bactericidal activity *via* downregulation of LPS-stimulated TLR signaling with subsequent reduction in interleukin (IL)-6 and IL-1ß gene expression.[36] Although triclosan monotherapy has poor activity against *Pseudomonas* sp., it has an adjuvant role in *Pseudomonas* biofilms when combined with oral aminoglycosides.[39] The activity of triclosan as an adjuvant therapy in those individuals with known biofilm disease is an area of potential future research.

Topical clindamycin

Clindamycin is one of the pillars of treatments for Hurley stage 1 and 2 HS as outlined in European guidelines.[6,40] It is also one of the few treatments to be examined in randomized controlled trials in

HS.[13] Overall, two randomized controlled trials have indicated the benefit of topical clindamycin over placebo[41,42] (Relative Risk [RR] = 0.72, 0.14–3.64) with a benefit in pain reduction, pustules, and inflammatory nodules, but no significant difference compared with tetracyclines.[42] Expert opinion considers it most beneficial for superficial pustules and solitary nodules, with recommendations to be used in the absence of deep-seated abscesses.[14,43] Clindamycin is a semisynthetic lincomycin derivative with a bacteriostatic effect through the inhibition of protein synthesis by 50S ribosomal binding.[44] It has broad Gram-positive cocci coverage and Gram-negative anaerobe coverage.[44] The direct antimicrobial effects of clindamycin may be relevant to HS, as *Porphyromonas* sp. and *Peptidophillus* sp. have high sensitivity to clindamycin.[36] Despite increasing reports of MRSA clindamycin resistance,[44] clindamycin has been shown to reduce the expression of virulence factors[45] including leucocidin, TSST01 and α-hemolysin. They also have direct effects on NF-κB and AP1 gene expression.[45] There are also *in vitro* reports of regulation of macrophage function, and response of neutrophils to chemokines.

Topical azelaic acid

Azelaic acid is a topical organic acid, traditionally used in acne vulgaris and acne rosacea[14,46] but has recently been recommended for use in HS as an adjuvant with topical clindamycin, particularly in pediatric cases.[14] Azelaic acid functions through antifungal and bacteriostatic properties[47] but also has well-documented anti-inflammatory properties through the modulation of peroxisome proliferator-activated receptor (PPAR)-γ activity and reduction in IL-6, TNF-α and IL-1ß.[48] HS is associated with altered PPAR-γ signaling[8] and the use of azelaic acid in early-stage HS lends credence to the thought of inflammation precedes follicular occlusion in HS.[8]

Topical retinoids

Retinoids are a class of compounds, chemically related to vitamin A. Topical and systemic retinoids are used in HS based upon their known anti-inflammatory properties[49,50] and the underlying presumption that HS as a disorder of follicular occlusion and hyperkeratinization.[51] No formal evaluation of the efficacy of topical retinoids in HS exists.[52] Despite the follicular occlusion paradigm being brought into question,[8] third-generation topical retinoids (adapalene, tazarotene) have significant anti-inflammatory activity through suppression of macrophage response to LPS stimulation, TNF-α, Toll Like Receptor 2 (TLR2) on monocytes, MMP1, MMP9 and VCAM1.[53] All of these pathways are documented in the pathogenesis of HS[8] The degree of cytokine reduction from topical retinoids is modest (IL-12 reduction from 840 pg/ml to 420 pg/ml) when compared with lesional HS cytokine levels.[8] Further clinical studies are needed to assess the clinical efficacy of topical retinoids in early-stage HS.

Resorcinol

Resorcinol is a benzenediol or phenol used as a chemical peeling agent as well as having antiseptic and antipruritic activity. The 15% resorcinol has been reported in one retrospective and one prospective study in HS.[54,55] Benefit was seen in patients with Hurley stage 1 and 2 disease, with twice daily application for 30 days with reduction in size of visible lesions, pain and erythema. The mechanism of action of resorcinol in HS is proposed to be keratolytic at the follicular infundibulum.[54] However, given the pharmacokinetics of the drug,[16] antiseptic properties dominate more than keratolytic activities at the concentrations used in HS. Resorcinol has potent antiseptic activity at a wide range of concentrations due to membrane damage resulting in K+ leakage, and direct uncoupling of oxidative phosphorylation[16] Some anti-inflammatory activities of resorcinol and related compounds have also been proposed, although are not described in detail.[56]

Intralesional therapies

Intralesional triamcinolone

Intralesional triamcinolone is an effective rescue therapy to reduce the inflammation, pain and swelling from active nodules and abscesses in HS.[57,58] Uncontrolled case series have identified a reduction in physician-assessed erythema, suppuration and size[57] as well as the rates of systemic antibiotic therapy.[58] However, a recent placebo-controlled trial suggested that intralesional steroids were not superior to placebo (saline) injections.[59] This brings into question our understanding of the

role of injections in symptom relief in HS as the benefit may be due to encourage spontaneous lesion rupture following injection rather than the pharmacological action of the steroid. Despite the lack of clarity regarding the mechanism of intralesional therapies in HS, oral corticosteroids are known to provide rapid relief in acute flares of HS and are beneficial as a low dose adjuvant with biologics and other immunomodulating agents. Therefore, the mechanisms of intralesional therapy in HS require further study.

Oral therapeutic agents

Anti-hyperglycemic agents

HS is positively associated with insulin resistance, even in multivariate analyses once adjusted for age sex and body mass index [2.51 (0.18) *versus* 1.92 (0.21); $p = 0.04$].[60,61] Hypoglycemic agents have been used in the management of HS and they have an important role as an adjuvant anti-inflammatory therapy and help to address underlying proinflammatory comorbidities in HS.[62] There is no evidence for their efficacy as a monotherapy[62] (Table 3). They may also be vital in addressing microbiome-mediated inflammatory stimuli, given the role of short chain fatty acid and bile salt mediators, such as those produced in HS by *Porphyromonas* sp. and *Peptinophillus* sp.,[63] as stimuli in other chronic inflammatory dermatoses.[64,65] The proposed mechanisms of action include the inhibition of mTORC1 activity in metformin,[66] leading to a decreased expression of IL-6, TNF-α[67] as well as downregulation of Th17 activity and the NLRP3 inflammasome.[68] Liraglutide, a glucagon-like 1 peptide analogue, increases the expression of transforming growth factor (TGF)-ß1 and decreases IL-17 expression.[69] Dipeptidyl peptidase IV (DPP4) inhibitors reduce the metabolism of glucagon-like peptide. Members of this class include sitagliptin, saxagliptin and linagliptin.[70] They have the additional benefit of slowing gastric emptying and promoting weight loss. Other anti-hyperglycemic agents including PPAR-γ agonists[71] (pioglitazone, rosiglitazone) are known to decrease insulin resistance and decrease levels of IL-6, as well as having antiproliferative activity.[71] Given the known role of PPAR-γ in sebaceous gland atrophy in HS,[8] modulation of PPAR activity may be a future therapeutic avenue.

Hormonal therapies

The evidence for the association between HS and estrogens, progestins, and androgen activity is contradictory. Whilst an epidemiological association with polycystic ovarian syndrome is known[72] a recent systematic review did not identify a consistent hormonal abnormality in HS patients,[73] although alterations in hormone levels are associated with disease flares.[73] This has been well documented in perimenstrual, perimenopausal and postpartum women experiencing painful disease flares. It has been proposed that end-organ (follicular) activity of sex hormones may play a role in disease pathogenesis,[74] however immunohistochemical findings show no evidence of dysregulated sex hormone receptors in the lesional skin of HS patients compared with healthy controls.[8] Despite this lack of evidence, a clinical benefit is seen in HS patients with anti-androgen therapy, including finasteride, spironolactone, and antiandrogenic progestogens (cyproterone acetate, chlormadinone acetate, drospirenone;[74,75] Table 3). It is acknowledged that variations in dosages and patient characteristics may confound the results of individual case reports; however, analysis of individual case reports in hormonal therapies is beyond the scope of this review. Sex hormones have immunomodulatory activity through their effects upon dendritic cells, T-cells maturation, differentiation, and suppression of the Th1 immune response.[76] Bimodal immune activity is dependent upon sex, the specific inflammatory site, the cytokine milieu and the endogenous sex hormone levels.[76,77] There is also evidence to suggest immune-modulating mechanisms of estrogens which are independent of canonical estrogen-response elements.[76,77] These include important immune pathways such as NF-κB, SP1 and AP1,[76,77] and signal transduction pathways including PI3K/AKT pathways[77] implicated in HS.[78] In line with other inflammatory rheumatological disorders, this may be mediated through estrogen metabolites (16-α estrogens), which are known to modulate local immune responses in arthritis and encourage the development of juxta-inflammatory adipose tissue and insulin resistance[77] both seen in HS. Hence, identification of 16-α estrogens is a potential biomarker candidate for individuals that may benefit from hormonal therapies in HS.

A number of case reports have documented clinical improvements in HS with finasteride, a

Table 3. Mechanism of action of oral agents (including oral antimicrobials) in HS. Canonical effects are compared with documented anti-inflammatory effects proposed in HS. The quality of evidence regarding their use in HS is also listed in the far-right column.

Oral therapy	Mechanism(s) of action		Quality of evidence[15]
	Canonical effect(s)	Documented anti-inflammatory effect(s)	
Metformin	Decreased mTORC1 activity	Decreased IL-6, TNF-α, TH17, NLRP3	C
DPP4 Inhibitors	Reduction of metabolism of GLP, slow gastric emptying, promote weight loss	Reduce adipose tissue associated TNF-α, IL-6 *via* NF-κB signaling	C
GP1 Analogues	Stimulate GP1 receptor		C
PPAR-γ Agonists	Stimulate PPAR-γ activity	Decrease IL-6 anti-proliferative activity	C
Oral Contraception	Estrogen-response elements on gene transcription	Dendritic cell, T-cell, possible role of 16-α estrogens NF-κB, AP-1, PI3/Akt pathway modulation	C
Finasteride	5-α reductase inhibitor	IGF-1 dependent: possibly increased activity in insulin resistance/diabetic patients	C
Spironolactone	Aldosterone antagonist	Reduction in TNF-α, IL-6, NOS	C
Oral antimicrobials			
Doxycycline	30S ribosomal inhibition	Decreased IL-1, IL-6, IL-8, TNF-α, chemotaxis, lipo-oxygenase inhibition, MMP inhibition, NF-κB signaling inhibition	B
Minocycline		Downregulation of LPS-stimulated TLR2 activity; upregulated TIMP-1	C
Erythromycin	50S ribosomal inhibition	Decreased IL-6, IL-8, TNF-α, GM-CSF activity. Downregulated AP-1 and NF-κB activity	C
Clindamycin			C
Rifampicin	Controversial: glucocorticoid receptor,	Reduced iNOS transcription, reduced NF-κB activity. Reduces TH17 differentiation	C
Ciprofloxacin	Inhibition of DNA gyrase and topoisomerase IV	Effects upon cAMP, NF-κB, AP-1, IL-8, IL-6 and gastrointestinal microbiome	C
Moxifloxacin		Reduction of IL-1ß, IL-8, TNF-α. Stabilization of IXb protein. Suppresses NF-κB signaling. Reduction in IL-17A	C

(Continued)

Table 3. (Continued)

Oral therapy	Mechanism(s) of action		Quality of evidence[15]
	Canonical effect(s)	Documented anti-inflammatory effect(s)	
Metronidazole	Inhibition of nucleic acid synthesis	Impacts on gastrointestinal microbiome	C
Ertapenem	Binding to Penicillin-binding proteins and disruption of cell wall synthesis	Reduction in IL-6, IL-12, TNF-α	C
Linezolid	Inhibition of protein synthesis initiation	Reduction in IL-1β, IL-6, IL-8, TNF-α. Possible ERK1/2 signaling modulation	C

AP-1, activator protein 1; cAMP, cyclic adenosine monophosphate; GLP, glucagon like peptide; GM-CSF, granulocyte-macrophage colony-stimulating factor; HS, hidradenitis suppurativa; IGF-1, Insulin Like Growth Factor- 1; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; NOS, nitric oxide synthase; PPAR, peroxisome proliferator-activated receptor; TLR2, Toll-like receptor; TNF, tumor necrosis factor.

5-α reductase antagonist.[79] Anecdotally, Clark[78] reports that finasteride is more effective in obese patients with HS. Given that 5-α reductase is insulin-like growth factor (IGF) 1-dependent, it is conceivable that finasteride may have increased benefits in individuals with insulin resistance or diabetes, although this has not been systematically investigated. Spironolactone is an aldosterone antagonist and has been associated with reduced inflammatory cytokine levels in various tissues,[80] as well as suppression of TNF-α, IL-6 and inhibition of NF-κB phosphorylation and nitric oxide synthesis,[80] although the precise mechanism in HS is not well described.

Antimicrobial therapies

Oral antibiotics

There is a steady shift away from considering HS as a disease of infectious etiology to a chronic inflammatory condition.[1,19] However the role of bacteria as either a driver of disease or a secondary bystander is unclear.[19,81] Alterations to the cutaneous microbiome do influence disease activity, but through an aberrant immune response rather than a traditional infectious response[82] Despite this, the role of antibiotics in HS is still seen by some patients and physicians as eliminating infection.[83] Examining the types of antibiotics used in HS (Table 3) reveals that the most effective options include therapies with significant anti-inflammatory effect (carbapenems, aminoglycosides).

Tetracyclines in HS, which have shown clinical efficacy also have no effect on HS bacteriological cultures, suggesting an effect independent of bactericidal activity.[84]

Tetracyclines

Tetracyclines are recommended for Hurley stage 1 or early-stage 2 disease[6,40] with one randomized controlled trial demonstrating no benefit compared with topical clindamycin.[42] Tetracyclines have well described anti-inflammatory activities aside from their antibacterial role through inhibition of the 30S ribosomal unit. Tetracyclines reduce IL-1, IL-6, TNF-α, and IL-8.[85] IL-8 is vital for inhibition of neutrophil chemotaxis, the inhibition of reactive oxygen species, MMPs and lipooxygenases.[85] This MMP-blocking ability is functional at submicrobial doses and in tetracyclines that have been altered to remove their antimicrobial activity.[85] Minocycline has the additional benefit of LPS-stimulated TLR2 inhibition and has similar reactive oxygen species scavenger activity to tocopherol (vitamin E). It also inhibits macrophage function through a reduction in oxidized lipids, upregulated tissue inhibitor of metalloproteinase (TIMP-1) and inhibits NF-κB signaling.[86]

Rifampicin

Rifampicin, in combination with clindamycin has been established as a beneficial treatment for HS.[6] The anti-inflammatory mechanism of action is controversial and may act *via* the corticosteroid receptor.[87] It also reduces the transcription of inducible nitric oxide synthase (iNOS) and competes with NF-κb for coactivator proteins and hence reduces NF-κB activity.[87] Rifampicin is also known to reduce TH17 differentiation and modulates T-cell responses.[88] From a proinflammatory standpoint it can also activate NF-κB and suppress PPAR-γ expression and activity.[87] This suppression of PPAR-γ only occurs in the presence of the proinflammatory cytokine milieu[88] although the clinical relevance of such data is unclear.

Clindamycin and erythromycin

Macrolide antibiotics function anti-microbially *via* the inhibition of the 50S ribosomal unit impairing bacterial protein synthesis.[89] Macrolides also alter the bacterial biofilm structure by altering polysaccharide synthesis. Other anti-inflammatory effects include the decreasing activity of TNF-α, IL-8, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in neutrophils and epithelial cells *via* the augmentation of activator protein 1 (AP-1) and NF-κB activity in the nucleus.[89] There is also some preliminary data suggesting augmentation of dendritic cell function with macrolides, with the effect varying between different forms of macrolide.[89] A combination of rifampicin-clindamycin has been a widely recommended treatment.[6] However, it has been recently proposed that rifampicin monotherapy may be sufficient for disease suppression and can be safely extended beyond the traditional 10-week mark, whilst the risks of *Clostridium difficile* infection remained elevated (coinciding with the use of clindamycin) during the 10 weeks of combined therapy.[90]

Moxifloxacin

Moxifloxacin is a fluoroquinolone antibiotic frequently used for the treatment of pneumonia[91] and is part of a recommended combination including rifampicin and metronidazole for disease control.[42] It has been demonstrated *in vitro* that moxifloxacin decreases activity of TNF-α, IL-8, IL-1ß *via* stabilization of the IXb protein and prevents translocation of NF-κB to the nucleus.[91,92] However, there is conflicting evidence regarding the clinical relevance of this anti-inflammatory activity. Animal models indicate that IL-1ß and IL-17A are reduced by moxifloxacin in the presence of viable bacteria, but not bacteria inactivated by heat.[93] Investigation in human models is needed to clarify the relevance of these results.

Metronidazole

As metronidazole is used as an adjuvant in therapy with moxifloxacin and rifampicin, the individual contribution of this drug to inflammation in HS is unclear. One unique aspect of metronidazole in comparison with other antibiotics used for the management of HS, is that is has well-documented impacts upon the gastrointestinal microbiome, resulting in metabolic dysregulation predisposing to obesity and insulin resistance.[64,65,94] Therefore in addition to the anti-inflammatory mechanisms of antibiotics in HS, microbiome alterations may also have indirect anti-inflammatory effects in HS.

Intravenous antibiotics

The intravenous antibiotics ertapenem and linezolid have been reported as highly effective in chronic recalcitrant HS[95,96] as a temporary bridge to definitive surgical management. Both drugs are known to have significant effects on the gut microbiota.[95,96] Despite its efficacy during the initial 6-week course of therapy, relapses and flares are commonly reported after treatment cessation, requiring ongoing intermittent dosing, which raises concerns regarding emerging resistance of *Pseudomonas* sp. and *Enterobacteriaceae* sp.[97] The shared anti-inflammatory activities of both agents include a reduction in IL-6, IL-12, TNF-α, as well as reduced activity of macrophages.[95,97,98] The risk of antibiotic resistance needs to be weighed against the efficacy of alternative anti-inflammatory therapies in the use of these therapies in HS.

Systemic immunomodulators and biologics

Given the recognized role of inflammation in the pathogenesis of HS,[8] systemic immunosuppression has been tested in HS with variable success (Table 4). The overall level of evidence for systemic immunosuppression in HS is low,[13] with

Table 4. Effects of systemic immunomodulators in HS including assessment of the quality of evidence in each therapy.

Systemic immunomodulator	Mechanism of action		Quality of evidence[15]
	Canonical effect	Proposed mechanism in HS	
Methotrexate	Dihydrofolate reductase inhibitor	Correction of FoxP3/Treg function leading to correction of TH17/Treg ratio	C
Cyclosporine	Calcineurin inhibitor	JNK p53 NFAT inhibitor, specific inhibitor of T-cells	C
Acitretin	ATRA prodrug: RXR/RAR nuclear transcription factors.	Alteration in AP-1, NF-κB transcription, Promotes conversion of naïve T-cells to Foxp3 regulatory T-cells	C
Isotretinoin			C
Dapsone	Reduction in superoxide production and neutrophil function	Reduction of neutrophil chemotaxis and oxidative damage	C
Apremilast	PDE4 inhibitor, reducing intracellular cAMP	Direct effect on T-cells dendritic cells, macrophages and monocytes, reducing IFN-γ and IL-2. Increases IL-10	B
INCB 57407	JAK1 inhibitor	Multiple sites of action including suppression of inflammatory activity through alteration in gene regulation and expression in keratinocytes and leukocytes	Trials ongoing NCT 03607487

cAMP, cyclic adenosine monophosphate; HS, hidradenitis suppurativa; IFN, interferon; IL, interleukin; NCT, ClinicalTrials.gov identifier; PDE4, phosphodiesterase 4; Treg, T regulator cell.

high-level evidence such as randomized controlled trials (RCTs) only available for etanercept[99] adalimumab,[100] infliximab,[101] anakinra,[102] apremilast[103] MABp1[104] and bermekimab (IFX-1).[105]

Colchicine

Colchicine is an anti-inflammatory agent used in the treatment of gout as well as autoinflammatory conditions including familial Mediterranean fever.[106] It functions *via* inhibition of tubulin polymerization, neutrophil function, suppression of NALP3 inflammasome, dendritic cell maturation as well as VEGF, S100A8, S100A9, NF-κB and Caspase 1.[106,107] It has demonstrated some benefit in prospective trials in HS,[107] however as in gout, it is limited by gastrointestinal side effects.

Methotrexate

Methotrexate has been reported in open-label studies but has not demonstrated any significant improvement in the degree of inflammation or frequency of flares in HS.[108] Methotrexate is useful in the prevention of autoantibodies in the setting of adalimumab and infliximab therapy;[109] however there is no indication in the literature that adjuvant methotrexate has a significant impact upon disease control in HS. Despite the fact that methotrexate has been shown to restore expression of FOXp3 and Treg function,[110] methotrexate is reported as of 'limited value' for the treatment of HS[108] with no patients in one prospective study demonstrating any improvement with the drug. This is suggestive that whilst Th17/Treg homeostasis is involved in the pathogenesis of HS,[67] either it is not the sole inflammatory mechanism, or only a specific patient

population may benefit from methotrexate therapy.

Cyclosporine

Cyclosporine therapy has been reported to result in substantial improvement in recalcitrant HS[111] however the true efficacy is difficult to discern given the co-administration of prednisolone and oral antibiotics in many cases.[112] The largest case series of 18 patients included only 2 patients who reported clinically significant improvement.[112] Putative mechanisms include the suppression of IL-2 and interferon (IFN)-γ *via* the known mechanisms of calcineurin inhibition in cyclosporine.[113] Where cyclosporine was co-administered with antibiotics it was unable to be elucidated whether the therapeutic effect was due to cyclosporine potentiating the effect of antibiotics (*via* CYP3A4) or the additive effects of both agents.

Acitretin and isotretinoin

Acitretin has been used in HS in various case series, with contradictory reports of success. A review by Blok and colleagues[114] reported an improvement rate of 73%; however, a recent series by Tan and colleagues reported no improvement with acitretin monotherapy.[115] A prospective study by Matusiak[116] showed up to half of patients treated showed some improvement in symptoms, with a large proportion of stage 1 and 2 patients demonstrating improvement.[116] Regarding the mechanisms of acitretin in HS, acitretin has been demonstrated to reduce the level of TH17 cells and serum IL-17 levels in psoriasis[114] and also contributes to re-stabilizing the Th17/Treg imbalance proposed to be central to inflammation in the disease.[67] The tolerability of treatment with retinoids has been hampered by the high dosages needed to sustain ongoing improvements.

Isotretinoin has been reported to have a moderate to significant improvement in younger, female patients with facial acne[114,117] however no clinical benefit has been seen in Hurley stage 3 patients. Its mechanism of action in acne has been reported to be mediated by multiple metabolites, including *via* the RXR-γ receptor leading to reduction in the size of the sebaceous glands. Given the known atrophy of sebaceous glands in established HS[8] it is more likely that it exerts an effect through immunomodulatory mechanisms. It is known that all-trans retinoic acid (of which isotretinoin is a prodrug) modulates the function of T-cells and monocytes,[118] particularly the induction of TH17 cells *via* IL-6.[67]

Dapsone

Dapsone, similarly to retinoids, provides improvement in mild HS and has no documented response in severe Hurley stage 3 disease.[119] Dapsone functions *via* anti-inflammatory and bacteriostatic properties.[120] Given the sulfone-sensitive nature of microbial flora in HS,[63] it is possible that the mechanism of action of dapsone may be contributed to by some antimicrobial effect. The anti-inflammatory effect of dapsone is mediated by suppression of superoxide production and it also downregulates the LPS-stimulated production of TNF-α and IL-8.[120] As both of these cytokines are prevalent in HS,[8] this could provide a mechanism for the partial response to dapsone. Dapsone is also known to reduce neutrophil chemotaxis[120] which may explain the additional effect in early-stage disease.

Apremilast

Apremilast is a phosphodiesterase 4 (PDE4) inhibitor currently used in psoriasis and psoriatic arthritis.[121] It has been explored as a potential treatment in other inflammatory conditions including HS, with one RCT[104] of 20 patients, with 53% achieving the hidradenitis suppurativa clinical response (HiSCR) at week 16. The mechanism of apremilast is the accumulation of intracellular cyclic adenosine monophosphate (cAMP) resulting in protein kinase A activation and the regulation of multiple transcription factors including activating transcription factor 1 (ATF-1), CREB-binding protein and NF-κB, which results in the decreased production of IFN-γ and IL-2. IL-10 is also increased with reduced stimulation of T-cells, monocytes and macrophages.[121,122] Given the diversity of the effects of apremilast this may be a promising avenue of investigation for the treatment of the inflammatory dysregulation in HS and shows a promising effect in well-established disease.

Vitamins, supplements and alternative treatments

Vitamins and supplements including zinc,[123] myo-inositol,[124] folic acid[124] and magnesium[124] have been reported in individual case reports in HS; however, there is no evidence to suggest

increased efficacy over antibiotic therapy.[124] There are also significant risks of toxicity and adverse effects with high dosages.[125] In instances where anti-inflammatory activity has been identified, no link to clinically significant response has been seen that could not be accounted for by placebo.[123,124] Overall, further investigation is needed into the mechanisms and role of alternative therapies in HS.

Monoclonal antibodies (biologics)

Monoclonal antibody therapy has revolutionized the treatment of chronic inflammatory disorders, such as psoriasis, rheumatoid arthritis and inflammatory bowel disease.[126] Adalimumab is currently the only US FDA-approved monoclonal biologic therapy for HS,[100] however a wide variety of monoclonal antibodies have been trialed as a therapy in HS (Table 5), including in current phase II clinical trials. Response rates vary between therapies, and given the specific targets of these drugs, this variation offers insights into important pathogenic pathways in HS. It may also confirm or refute the concept of pathogenic heterogeneity in HS.[127]

Elevated levels of TNF-α, IL-17, IL-1 and C5a have been identified in lesional tissue of HS patients[8] and this has been the justification for selective targeting of these inflammatory pathways. TNF-α, as a nonspecific inflammatory cytokine, has inferior clearance and response rates to other therapies such as IL-17 and IL-23 inhibitors in psoriasis[128] and the hope is that, similarly to psoriasis, novel HS-specific therapeutic targets will be identified for which monoclonal antibodies can be developed. TNF-α has broad anti-inflammatory effect including modulating the activity of dendritic cells, monocytes and neutrophils.[128]

The role of IL-17 inhibition in HS is currently under investigation in multiple phase II clinical trials (Table 5). The underlying premise behind IL-17 inhibition includes the presence of Th17 cells in HS lesional tissue,[8] the role of IL-22, IL-17 and IL-23 in promoting the epidermal hyperplasia seen in HS[8] and evidence from case reports of efficacy of IL-17 blockade in HS.[129,130,131] Strong IL-17 signals are seen more in moderate and severe disease,[8] so IL-17 may be beneficial in patients where TNF-α therapy has failed. A concern with the use of IL-17 antagonism in HS, however, is the association of HS with inflammatory bowel disease (IBD)[131] and the inefficacy (and paradoxical worsening of disease) with IL-17 blockade in RCTs of IBD.[132] Interestingly, genetic studies identified the absence of a minor allele (TNF-like ligand 1A) which was associated with a lack of response in these patients.[132] Therefore, if IL-17 blockade does demonstrate an effect in HS, there may be pharmacogenomic indicators which may predict benefit in patients with HS and IBD. This would be an interesting area of further research.

Novel agents including IFX-1,[104] targeting C5a anaphylatoxin, are purported to indirectly suppress TNF-α activity in HS. An RCT including 20 patients demonstrated response rates of 60% compared with 10% for placebo.[104] C5a signaling is also involved in IL-17 and IL-23 signaling through the modulation of dendritic cell activity.[133,134] However, the unaffected section of the complement cascade involving C5b-9 and membrane attack complex activation leads to multiple signal transduction activity including PI3K/Akt activation, STAT3 phosphorylation and cDC2 activation.[132] The cDC2 activity mediates TNF-α activity as well as the modulation of transforming growth factor (TGF)-ß activity but does not block IL-22 production, which mediates Th17 activity.[133,134] This mechanism may explain persistent inflammation in patients not responsive to IFX-1 and C5a blockade,[104] but also gives hope that IFX-1 may be more effective in patients with more advanced (Hurley stage 3) disease with fistulae, hypertrophic scarring and high TGF-ß activity.[8]

IL-1 blockade has been trialed in HS with the use of anakinra,[102] an IL-1R antagonist and more recently with bermekimab[105] a fully human anti-IL-1α antagonist. Both drugs have been investigated in RCTs (Figure 3) with response rates of 78%[102] and 60%[105] respectively. Cytokines of the IL-1 family (IL-1α, IL-1ß, IL-1R and IL-33) are important mediators of T-cell recruitment in inflammatory skin disease.[8,135] It also has significant potential for activation of fibroblasts which may be involved in the development of scarring and fistulae in HS.[8,135] The disparate rates in clinical response between anakinra and bermekimab also suggests that other members of the IL-1 family (such as IL-18 or IL-33) may be involved in HS which are targeted by anakinra but not by bermekimab.[135]

Table 5. Monoclonal antibodies reported in HS including drugs under investigation.

Monoclonal antibody	Mechanism(s) of action		Quality of evidence[15]
	Therapeutic target	Proposed mechanism in HS	
Adalimumab	TNF-α	Reduction in TNF-α associated inflammation as well as keratinocyte-mediated feed-forward mechanisms.	A
Etanercept	TNF-α		B
Infliximab	TNF-α		B
Anakinra	IL-1R	Interruption of keratinocyte-mediated feed-forward mechanisms as well as microbiome-associated inflammatory drive.	B NCT 01516749 NCT 01558375
Bermekimab (MABp1)	IL-1α		B NCT 02643654
Secukinumab	IL-17A	Preferential suppression of keratinocyte-induced feed-forward mechanisms and correction of Th17/Treg dysfunction	Trials ongoing NCT 03099980
Bimikizumab	IL-17A / IL-17F		Trials ongoing NCT 03248531
Ixekizumab	IL-17A		C (case reports)
Ustekinumab	IL-12 /IL-23 p40 subunit		Trials ongoing NCT 01704534
Guselkumab	IL-23		Trials ongoing NCT 03628924
IFX-1	C5a	Indirect suppression of TNF-α *via* upstream pathways	Trials ongoing (NCT 03001622)
Efalizumab	LFA-1	Interruption of ICAM-1-mediated inflammation No improvement in prospective trial ($n = 5$)	NCT 00134134
Drugs under investigation			
MEDI8968	IL-1 receptor I inhibitor	Interruption of keratinocyte-mediated feed-forward mechanisms as well as microbiome-associated inflammatory drive.	Trials ongoing NCT 01838499
CJM112	IL-17A inhibitor	Preferential suppression of keratinocyte-induced feed-forward mechanisms and correction of Th17/Treg dysfunction	Trials ongoing NCT 02421172

HS, hidradenitis suppurativa; IL, interleukin; NCT, ClinicalTrials.gov identifier; PDE4, phosphodiesterase 4; TNF, tumor necrosis factor; Treg, T regulator cell.

Ustekinumab has been reported in a case reports[136] and an open-label study[137] as a therapy for HS, and elevation of IL12p40 has been identified in HS.[8] Response rates (HiSCR) are reported as 47% with a positive response associated with low levels of LTA4H (leukotriene A4 hydrolaze)[137] suggesting the activity of ustekinumab is more beneficial in the presence of active leukocyte activity,[138] seen in early

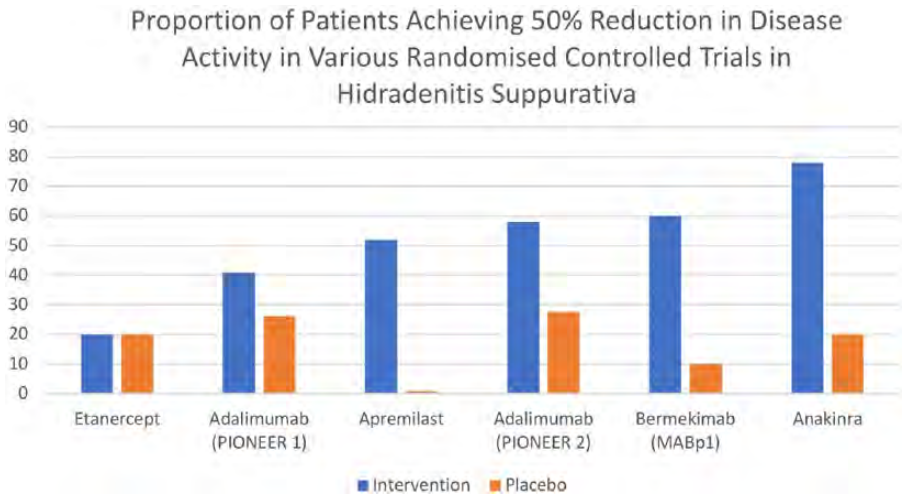

Figure 3. Reported proportion of response in different RCTs in HS.
HS, hidradenitis suppurativa; RCT, randomized controlled trial.

rather than advanced disease. Efalizumab (Raptiva®) has been investigated in a small cohort study[139] of five women with moderate to severe, treatment-refractory HS. None of the individuals reported improvement of their symptoms.[139] Efalizumab functions through the blocking the actions of LFA-1 (leukocyte function associated antigen 1), including ICAM-1 binding, ICAM-1 has been identified as involved in the inflammatory mechanisms of HS[8] and is suggested to be involved in *Porphyromonas* and *Peptinophillus*-mediated inflammatory stimulation, including in the development of fistulae. Blockage of ICAM-1 binding to leukocytes may potentiate the epithelial activity of ICAM-1 leading to increases in epithelial-associated inflammation, accounting for the worsening of disease seen in these patients.[139]

Other medications currently under investigation in clinical trials include JAK1 inhibitors, which, through IL-6 uncoupling, may ameliorate IL-22-mediated inflammation as well as potentially the progression of scarring as is seen in other disorders including chronic GvHD.[140] However, given the contribution of Th1 and Th17 to inflammation in HS,[8] it may be that combination of JAK1 and JAK2 inhibition may be required in HS.[141]

Translating therapy into disease mechanisms

The common pathways implicated in topical, systemic and immunomodulatory therapies are illustrated in Figure 2. Significant differences exist between the agents and implicated pathways in therapy for mild as opposed to severe disease. This is an expression of the possible dichotomy in inflammatory pathways (and hence varying responses to treatment) that exists in HS.

A common theme in multiple agents recommended for use in mild to moderate disease is the augmentation of the NF-κB pathway as well as other inflammatory cytokines including TNF-α and IL-6, whether this be through topical or oral antibiotic therapy. This nonspecific inflammatory pathway, as well as the favorable rates of remission in mild HS, suggest that the disease in this stage is comparable to the chronic, relapsing inflammatory disorders including psoriasis and atopic dermatitis, which we are able to treat effectively. Continuous therapy can control the disease, but therapy must be balanced against adverse effects, patient preference and potential long-term sequelae. The role of 16-α estrogens as a potential biomarker for the utility of hormonal therapy in HS is also an area requiring further study.

The role of bacteria in HS is still controversial; however, what is agreed upon is that the inflammatory component of HS is likely to be an aberrant response to a dysbiotic cutaneous microbiome.[19,63] In early disease, antibacterial actions may have an effect upon the disease process, but the evidence to date suggests that the majority of effects are due to anti-inflammatory alterations. The bacteriostatic effect of antibacterial treatments may also indicate

that bacterial virulence factors (which are not eliminated by bacteriostatic effects) may also play a role in the pathogenesis of disease. There is a moderate impact on biofilm formation and prevalence, and the incomplete elimination of biofilms may contribute to disease recurrence along with the presence of a chronic feed-forward inflammatory drive. Despite this, the only proven major benefit of antibacterial therapies are the prevention of secondary colonization of impaired skin. These results are in direct conflict with the follicular occlusion paradigm in HS and implies that inflammation precedes occlusion, as in acne vulgaris. This is also supported by immunohistochemical studies of HS.[8]

When HS presents (or progresses) to moderate and severe disease (presence of scarring, fistulae and draining sinuses), therapeutic escalation to systemic immunomodulatory therapies and intravenous antibiotic therapies commonly ensues. It is difficult to untangle the role of the microbiome in moderate to severe HS, however, one hypothesis that high dose antibiotics, through profound changes in the gastrointestinal microbiome, may influence systemic inflammation, is captivating. The (albeit limited) benefit of systemic agents such as retinoids suggest that NF-κB and AP-1 transcription factors[142,143] still play a central role in the inflammatory cascade of more advanced HS. However, the lack of efficacy of other therapies including hormonal therapies, methotrexate and cyclosporine suggest that other self-perpetuating inflammatory mechanisms are involved.

Studies involving monoclonal antibodies provide the greatest insight into what these specific mechanisms may be. The varying levels of clinical efficacy compared with placebo (Figure 3) bolsters the suggestion that differential inflammatory pathways may be involved in different presentations of disease. Within studies, particularly with adalimumab,[100] the rates of efficacy were reduced for Hurley stage 3 patients than Hurley stage 2 patients. This is in contrast to bermekimab[105] and anakinra,[102] where response rates are highest; and the proportion of Hurley stage 3 patients are significantly higher. It is unclear whether IL-17 blockade will have similar results to TNF-α inhibition in HS (with greater benefit in mild disease as opposed to severe disease), or whether due to the augmentation in epithelial hyperplasia, keratinocyte-mediated feed-forward mechanisms may be interrupted, leading to similar response rates to IL-1 blockade (with greater utility in severe disease). Small molecule targets such as apremilast and JAK inhibition have the potential to be useful monotherapies and adjuvants and further clinical trials are sorely needed.

The significant placebo rates (up to 30%) in HS RCTs serve as a caveat to the interpretation of small degrees of clinical improvement in HS. Due to the lack of controlled, untreated natural history studies in HS, the rates of spontaneous resolution of lesions are difficult to discern in comparison with other chronic inflammatory skin diseases, such as atopic dermatitis and psoriasis. An additional complicating factor is the proinflammatory contribution of metabolic comorbidities (such as diabetes and obesity).[8] Subgroup analysis in existing studies is unreliable due to small patient numbers, however it is feasible that specific therapies may be more beneficial in HS patients with specific comorbidities due to the specific cytokine stimulation caused by other comorbid conditions present in these patients.

Conclusion

Examining the therapeutic mechanisms in treatments for HS has identified a potential dichotomy in inflammatory processes between mild to moderate and moderate to severe disease. NF-κB, TNF-α and other innate immune mechanisms are strongly represented in treatments which are effective in mild to moderate disease in the absence of scarring or draining fistulae (Hurley stage 1 and Hurley stage 2 disease); however, complex feed-forward mechanisms in severe disease (Hurley stage 2 to Hurley stage 3) respond to IL-1 inhibition but are less likely to respond to innate immune inhibition (through NF-κB or TNF-α) alone. It is unclear whether IL-17 inhibition will parallel TNF-α or IL-1 inhibition in effect; however, it is plausible that small molecule targets may provide an effective new strategy. It is also plausible that personalized treatment regimens based upon comorbidities and genetic variants will be essential to identify the most effective therapy for HS patients.

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References

1. Hoffman LK, Ghias MH and Lowes MA. Pathophysiology of Hidradenitis Suppurativa. *Semin Cutan Med Surg* 2017; 36: 47–54.
2. Ingram JR, Jenkins-Jones S, Knipe DW, *et al.* Population-based clinical practice research datalink study using algorithm modelling to identify the true burden of hidradenitis suppurativa. *Br J Dermatol* 2018; 178: 917–924.
3. Frew JW, Vekic DA, Woods JA, *et al.* A systematic review and critical evaluation of reported pathogenic sequence variants in hidradenitis suppurativa. *Br J Dermatol* 2017; 177: 987–998.
4. Kirby JS, Miller JJ, Adams DR, *et al.* Healthcare utilization patterns and costs for patients with hidradenitis suppurativa. *JAMA Dermatol* 2014; 150: 937–944.
5. Saunte DM, Boer J, Stratigos A, *et al.* Diagnostic delay in hidradenitis suppurativa is a global problem. *Br J Dermatol* 2015; 173: 1546–1549.
6. Zouboulis CC, Desai N, Emtestam L, *et al.* European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; 29: 619–644.
7. Fotiadou C, Vakirlis E and Ionnides D. Spotlight on adalimumab in the treatment of active moderate-to-severe hidradenitis suppurativa. *Clin Cosmet Invest Dermatol* 2016; 9: 367–372.
8. Frew JW, Hawkes JE and Krueger JG. A systematic review and critical evaluation of inflammatory cytokine associations in hidradenitis suppurativa. *F1000Res* 2018.
9. Riis PT, Thorlacius LR and Jemec GB. Investigational drugs in clinical trials for hidradenitis suppurativa. *Expert Opin Investig Drugs* 2018; 27: 43–53.
10. Maarouf M, Clark AK, Lee DE, *et al.* Targeted treatments for hidradenitis suppurativa: a review of the current literature and ongoing clinical trials. *J Dermatolog Treat* 2018; 29: 441–449.
11. Frew JW, Hawkes JE and Krueger JG. A systematic review and critical evaluation of immunohistochemical associations in hidradenitis suppurativa [version 1; referees: awaiting peer review]. *F1000Research* 2018; 7: 1923, https://doi.org/10.12688/f1000research.17268.1
12. Pesce A, Capuzzo G, Cammisuli B, *et al.* Pilonidal disease, hidradenitis suppurativa and follicular occlusion syndrome: a diagnostic challenge. *Eur Rev Med Phamacol Sci* 2018; 22: 4755–4756.
13. Ingram JR, Woo PN, Chua SL, *et al.* Interventions for hidradenitis suppurativa. *Cochrane Database Syst Rev* 2015; 10: CD010081.
14. Deckers IE and Prens EP. An update on medical treatment options for hidradenitis suppurativa. *Drugs* 2016; 76: 215–229.
15. Robinson JK. Systematic reviews: grading recommendations and evidence quality. *Arch Dermatol* 2008; 144: 97–99.
16. McDonnell G and Russell D. Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Reviews* 1999; 12: 147–179.
17. Bonez PC, Filippidos C, Alves S, *et al.* Chlorhexidine activity against bacterial biofilms. *Am J Infect Control* 2013; 41: e119–e122.
18. Fitzgerald KA, Davies A and Russell AD. Bacterial uptake of 14C-chlorhexidine diacetate and 14C-benzyl alcohol and the influence of phenoxyethanol and azolectin: studies with gram-negative bacteria. *Microbios* 1992; 70: 77–91.

19. Guet-Revillet H, Jais JP, Ungeheuer MN, *et al.* The microbiological landscape of anaerobic infections in hidradenitis suppurativa: a prospective metagenomic study. *Clin Infec Dis* 2017; 65: 282–291.
20. Ingram JR and McPhee M. Management of hidradenitis suppurativa: a UK survey of current practice. *Br J Dermatol* 2015; 173: 1070–1072.
21. Bigliardi PL, Alsagoff SAL, El-Kafrawi HY, *et al.* Povidone iodine in wound healing: a review of current concepts and practices. *Int J Surgery* 2017; 44: 260–268.
22. Chandler CJ and Segel IH. Mechanism of the antimicrobial action of pyrithione: effects on membrane transport, ATP levels, and protein synthesis. *Antimicrob Agents Chemother* 1978; 14: 60–68.
23. Kitamura H, Morikawa H, Kamon H, *et al.* Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. *Nat Immunol* 2006; 7: 971–977.
24. Lamore SD and Wondrak GT. Zinc pyrithione impairs zinc homeostasis and upregulates stress response gene expression in reconstructed human epidermis. *BioMetals* 2011; 24: 875–890.
25. Yoon KS, Youn N, Gu H, *et al.* Estrogenic activity of zinc pyrithione: an in vivo and in vitro study. *Environ Health Toxicol* 2017; 32: e2017004.
26. Perumal PK, Wand ME, Sutton JM, *et al.* Evaluation of the effectiveness of hydrogen peroxide-based disinfectants on biofilms formed by Gram-negative pathogens. *J Hosp Infect* 2014; 87: 227–233.
27. Lajevardi SS and Abeysinghe J. Novel technique for management of axillary hidradenitis suppurativa using setons. *Case Rep Surg* 2015; 2015: 369657.
28. Banerjee S, Zmijewski JW, Lorne E, *et al.* Modulation of SCFBetaTrCP dependent IkBa ubiquination by hydrogen peroxide. *J Biol Chem* 2010; 285: 2665–2675.
29. Wittmann C, Chockley P, Singh SK, *et al.* Hydrogen peroxide in inflammation: messenger, guide, and assassin. *Adv Hematol* 2012; 2012: 541471.
30. Huang JT, Abrams M, Tlougan B, *et al.* Treatment of Staphylococcus aureus colonization in atopic dermatitis decreases disease severity. *Pediatrics* 2009; 123: e808–e814.
31. Leung TH, Zhang LF, Wang J, *et al.* Topical hypochlorite ameliorates NF-κB-mediated skin diseases in mice. *J Clin Invest* 2013; 123: 5361–5370.
32. Eriksson S, van der Plas MJA, Morgelin M, *et al.* Antibacterial and antibiofilm effects of sodium hypochlorite against Staphylococcus aureus isolates derived from patients with atopic dermatitis. *Br J Dermatol* 2017; 117: 513–521.
33. Epstein AK, Pokroy B, Seminara A, *et al.* Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration. *Proc Natl Acad Sci U S A* 2011; 108: 995–1000.
34. Knowlden SA, Yoshida T, Perez-Nazario N, *et al.* Bleach baths promote early induction of inflammatory pathway genes with no effect on skin bacterial dysbiosis in AD subjects [abstract]. *J Invest Dermatol* 2017; 137: S50.
35. Barros SP, Wirojchanasak S, Barrow DA, *et al.* Triclosan inhibition of acute and chronic inflammatory gene pathways. *J Clin Periodontol* 2010; 37: 412–418.
36. Lubarsky HV, Gerbersdorf SU, Hubas C, *et al.* Impairment of the bacterial biofilm stability by triclosan. *PLoS One* 2012; 7: e31183.
37. Hessam S, Sand M, Meier NM, *et al.* Combination of oral zinc gluconate and topical triclosan: an anti-inflammatory treatment modality for initial hidradenitis suppurativa. *J Dermatol Sci* 2016; 84: 197–202.
38. Maiden MM, Agostinho Hunt AM, Zachos MP, *et al.* Triclosan is an aminoglycoside adjuvant for the eradication of Pseudomonas aeruginosa biofilms. *Antimicrob Agents Chemother* 2018; 62: pii: e00146–18.
39. Gulliiver W, Zouboulis CC, Prens E, *et al.* Evidence-based approach to treatment of hidradenitis suppurativa/acne inversa, based on the European guidelines for hidradenitis suppurativa. *Rev Endocr Metab Disord* 2016; 17: 343–351.
40. Clemmensen OJ. Topical treatment of hidradenitis suppurativa with clindamycin. *Int J Dermatol* 1983; 22: 325–328.
41. Jemec GB and Wendelboe P. Topical clindamycin versus systemic tetracycline in the treatment of hidradenitis suppurativa. *J Am Acad Dermatol* 1998; 39: 971–974.
42. Zouboulis CC, Bechara FG, Dickinson-Block JL, *et al.* Hidradenitis suppurativa/acne inversa: a practical framework for treatment optimization – systematic review and recommendations from

the HS ALLIANCE working group. *J Eur Acad Dermatol Venereol*. Epub ahead of print 23 October 2018. DOI: 10.1111/jdv.15233.

43. Hodille E, Badiou C, Bouveyron C, *et al*. Clindamycin suppresses virulence expression in inducible clindamycin-resistant Staphylococcus Aureus strains. *Ann Clin Microbiol Antimicrob* 2018; 17: 38.

44. Altenberg J, de Graaf CS, van de Werth TS, *et al*. Immunomodulatory effects of macrolide antibiotics - Part 1: biological mechanisms. *Respiration* 2011; 81: 67–74.

45. Sieber MA and Hegel JK. Azelaic acid: properties and mode of action. *Skin Pharmacol Physiol* 2014; 27(Suppl. 1): 9–17.

46. Charnock C, Brudeli B and Klaveness J. Evaluation of the antibacterial efficacy of diesters of azelaic acid. *Eur J Pharm Sci* 2004; 21: 589–596.

47. Mastrofrancesco A, Ottaviani M, Aspite N, *et al*. Azelaic acid modulates the inflammatory response in normal human keratinocytes through PPARγ activation. *Exp Dermatol* 2010; 19: 813–820.

48. Dreno B, Gollnick HPM, Kang S, *et al*. Understanding innate immunity and inflammation in acne: implications for management. *J Eur Acad Dermatol Venereol* 2015; 29(Suppl. 4): 3–11.

49. Bikowski JB. Mechanisms of the comedolytic and anti-inflammatory properties of topical retinoids. *J Drugs Dermatol* 2005; 4: 41–47.

50. Canavan TN, Chen E and Elewski BE. Optimizing non-antibiotic treatment for patients with acne: a review. *Dermatol Ther (Heidelb)* 2016; 6: 555–578.

51. Gill L, Williams M and Hamzavi I. Update on hidradenitis suppurativa: connecting the tracts. *F1000Prime Rep* 2014; 6: 112.

52. Schmidt N. Tretinoin: a review of its anti-inflammatory properties in the treatment of acne. *J Clin Aesthet Dermatol* 2011; 4: 22–29.

53. Boer J and Jemec GB. Resorcinol peels as a possible self-treatment of painful nodules in hidradenitis suppurativa. *Clin Exp Dermatol* 2010; 35: 36–40.

54. Pascual JC, Encabo B, Ruiz de Apodaca RF, *et al*. Topical 15% resorcinol for hidradenitis suppurativa: AN uncontrolled prospective trial with clinical and ultrasonographic follow-up. *J Am Acad Dermatol* 2017; 77: 1175–1178.

55. Lightowler JE and Rylance HJ. On the anti-inflammatory activity of some substituted phenolic compounds. *Br J Pharmacol Chemother* 1964; 22: 221–227.

56. Riis PT, Boer J, Prens EP, *et al*. Intralesional triamcinolone for flares of hidradenitis suppurativa (HS): a case series. *J AM Acad Dermatol* 2016; 75: 1151–1155.

57. Kraft JN and Searles GE. Hidradenitis suppurativa in 64 female patients: retrospective study comparing oral antibiotics and antiandrogen therapy. *J Cutan Med Surg* 2007; 11: 125–131.

58. Wong D, Walsh S and Alhusayen R. Low-dose systemic corticosteroid treatment for recalcitrant hidradenitis suppurativa. *J AM Acad Dermatol* 2016; 75: 1059–1062.

59. Fajenbaum K. Intralesional triamcinolone, a standard of care treatment for acute hidradenitis suppurativa, does not prove to be superior to placebo. In: *2nd annual symposium on hidradenitis suppurativa*, Hotel St Regis, Detroit, MI, USA, 4–5 November 2017.

60. Vilanova I, Hernandez JL, Mata C, *et al*. Insulin resistance in hidradenitis suppurativa: a case control study. *J Eur Acad Dermatol Venereol* 2018; 32: 820–824.

61. Verdolini R, Clayton N, Smith A, *et al*. Metformin for the treatment of hidradenitis suppurativa: a little help along the way. *J Eur Acad Dermatol Venereol* 2013; 27: 1101–1108.

62. Ring HC, Thorsen J, Saunte DM, *et al*. The follicular skin microbiome in patients with hidradenitis suppurativa and healthy controls. *JAMA Dermatol* 2017; 153: 897–905.

63. Kim CH. Immune regulation by microbiome metabolites. *Immunology* 2018; 154: 220–229.

64. Tan JK, McKenzie C, Marino E, *et al*. Metabolite-sensing G protein coupled receptors – facilitators of diet-related immune regulation. *Ann Rev Immunol* 2017; 35: 371–402.

65. Valeria de Vita Melnik BC. mTORC1 at the crossroad of metabolism and immunity in hidradenitis suppurativa. *J Eur Acad Dermatol Venereol*. Epub ahead of print 5 October 2018. DOI: 10.1111/jdv.15270.

66. Melnik BC, John SM, Chen W, *et al*. T helper 17 cell/regulatory T-cell imbalance in

hidradenitis suppurativa/acne inversa: the link to hair follicle dissection, obesity, smoking and autoimmune conditions. *Br J Dermatol* 2018; 179: 260–272.

67. Akdogan N, Alli N and Uysal PI. Visfatin and insulin levels and cigarette smoking are independent risk factors for hidradenitis suppurativa: a case control study. *Arch Dermatol Res*. Epub ahead of print 6 October 2018. DOI: 10.1007/s00403–018–1867.
68. Insuela DRB and Carvalho VF. Glucagon and glucagon like peptide-1 as novel anti-inflammatory and immunomodulatory compounds. *European J Pharmacol* 2017; 812: 64–72.
69. Nauck M. Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. *Diabetes Obes Metabol* 2016; 18: 203–216.
70. Dozsa A, Dezso B, Toth BI, *et al*. PPAR gamma-mediated and arachidonic acid dependent signaling is involved in differentiation and lipid production of human sebocytes. *J Invest Dermatol* 2014; 134: 910–920.
71. Garg A, Neuren E and Strunk A. Hidradenitis suppurativa is associated with polycystic ovary syndrome: a population-based analysis in the United States. *J Invest Dermatol* 2018; 138: 1288–1292.
72. Riis PT, Ring HC, Themstrup L, *et al*. The role of androgens and estrogens in hidradenitis suppurativa: a systematic review. *Acta Derm Croat* 2016; 24: 239–249.
73. Karagiannidis I, Nikolakis G, Sabat R, *et al*. Hidradenitis suppurativa/acne inversa: an endocrine skin disorder? *Rev Endocr Metab Disord* 2016; 17: 335–341.
74. Karagiannidis I, Nikolakis G and Zouboulis CC. Endocrinological aspects of hidradenitis suppurativa. *Derm Clin* 2016; 34: 45–49.
75. Bereshchenko O, Bruscoli S and Riccardi C. Glucocorticoids, sex hormones, and immunity. *Front Immunol* 2018; 9: 1332.
76. Straub RH. Interaction of the endocrine system with inflammation: a function of energy and volume regulation. *Arthritis Res Ther* 2014; 16: 203.
77. Xiao X, He Y, Li C, *et al*. Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signaling pathways. *Br J Dermatol* 2016; 174: 522–532.
78. Clark AK, Quinonez RL and Saric S. Sivamani hormonal therapy for hidradenitis suppurativa: review. *Dermatol Online J* 2017; 23.
79. Kato Y, Kamiya H, Koide N, *et al*. Spironolactone inhibits production of proinflammatory mediators in response to lipopolysaccharide via inactivation of nuclear factor-κB. *Immunopharmacol Immunotoxicol* 2014; 36: 237–241.
80. Zhang L, Hao JB, Ren LS, *et al*. The aldosterone receptor antagonist spironolactone prevents peritoneal inflammation and fibrosis. *Lab Invest* 2014; 94: 839–850.
81. Saunte DML and Jemec GBE. Hidradenitis suppurativa: advances in diagnosis and treatment. *JAMA* 2017; 318: 2019–2032.
82. Naik HB, Nassif A, Ramesh MS, *et al*. Are bacteria infectious pathogens in hidradenitis suppurativa (HS)? Debate at the symposium for hidradenitis suppurativa advances meeting, November 2017. *J Invest Dermatol*. Epub ahead of print 8 November 2018. DOI: 10.1016/j.jid.2018.09.036.
83. Zaaroura H, Geffen Y, Bergman R, *et al*. Clinical and microbiological properties of Staphylococcus lugdunensis skin infections. *J Dermatol* 2018; 45: 994–999.
84. Tilakaratne A and Soory M. Anti-inflammatory actions of adjunctive tetracyclines and other agents in periodontitis and associated comorbidities. *Open Dent J* 2014; 8: 109–124.
85. Sun J, Shigemi H, Tanaka Y, *et al*. Tetracyclines downregulate the production of LPS-included cytokines and chemokines in THP-1 cells via ERK, p38 and nuclear factor kB signaling pathways. *Biochem Biophys Res* 2015; 4: 397–404.
86. Schmidt KE, Kuepper JM, Schumak B, *et al*. Doxycycline inhibits experimental cerebral malaria by reducing inflammatory immune reactions and tissue-degrading mediators. *PLos One* 2018; 13: e0192717.
87. Yuhas Y, Berent E, Ovadiah H, *et al*. Rifampin augments cytokine-induced nitric oxide production in human alveolar epithelial cells. *Antimicrob Agents Chemother* 2006; 50: 396–398.
88. Ma K, Chen X, Chen JC, *et al*. Rifampicin attenuates experimental autoimmune

encephalomyelitis by inhibiting pathogenic Th17 cells responses. *J Neurochem* 2016; 139: 1151–1162.

89. Altenberg J, de Graaf CS, van der Werf TS, *et al*. Immunomodulatory effects of macrolide antibiotics – part 1: biological mechanisms. *Respiration* 2011; 81: 67–74.

90. Albrecht J, Barbaric J and Nast A. Rifampin alone may be enough. Is it time to abandon the oral clindamycin-rifampicin combination for hidradenitis suppurativa? *Br J Derm*. Epub ahead of print 15 November 2018. DOI:10.1111/bjd.17422.

91. Weiss T, Shalit I, Blau H, *et al*. Anti-Inflammatory effects of moxifloxacin on activated human monocytic cells: inhibition of NF-kB and mitogen activated protein kinase activation and of synthesis of pro-inflammatory cytokines. *Antimicrob Agents Chemother* 2004; 48: 1974–1982.

92. Choi JH, Song MJ, Kim SH, *et al*. Effect of moxifloxacin on production of proinflammatory cytokines from human peripheral blood mononuclear cells. *Antimicrob Agents Chemother* 2003; 47: 3704–3707.

93. Beisswenger C, Honecker A, Kamyschnikow A, *et al*. Moxifloxacin modulates inflammation during murine pneumonia. *Respir Res* 2014; 15: 82.

94. Rodrigues RR, Greer RL, Dong X, *et al*. Antibiotic induced alterations in gut microbiota are associated with changes in glucose metabolism in healthy mice. *Front Microbiol* 2017; 8: 2306.

95. Chahine AA, Nahhas AF, Braunberger TL, *et al*. Ertapenem rescue therapy in hidradenitis suppurativa. *JAAD Case Rep* 2018; 4: 482–483.

96. Yoshizawa S, Tateda K, Saga T, *et al*. Virulence suppressing effects of linezolid on methicillin-resistant Staphylococcus aureus: possible contribution to early defervescence. *Anti Microb Agents Chemother* 2012; 56: 1744–1748.

97. Mendes-Bastos P, Martorell A and Magina S. Ertapenem for the treatment of hidradenitis suppurativa: how much do we need it? *Actas Dermosifiliogr* 2018; 109: 582–583.

98. Bode C, Muenster S, Diedrich B, *et al*. Linezolid, vancomycin and daptomycin modulate cytokine production, Toll-like receptors and phagocytosis in a human in vitro model of sepsis. *J Antibiotics* 2015; 68: 485–490.

99. Adams D, Yankura JA, Fogelberg AC, *et al*. Treatment of hidradenitis suppurativa with etanercept injection. *Arch Dermatol* 2010; 146: 501–504.

100. Kimball AB, Okun MM, Williams DA, *et al*. Two phase 3 trials of adalimumab for hidradenitis suppurativa. *N Engl J Med* 2016; 375: 422–434.

101. Grant A, Gonzales T, Montgomery MO, *et al*. Infliximab therapy for patients with moderate to severe hidradenitis suppurativa: a randomized, double-blind placebo-controlled crossover trial. *J Am Acad Dermatol* 2010; 62: 205–217.

102. Tzanetakou V, Kanni T, Giatrakou S, *et al*. Safety and efficacy of anakinra in severe hidradenitis suppurativa a randomized clinical trial. *JAMA Dermatol* 2016; 152: 52–59.

103. Vossen ARJV, van Doorn MBA, van der Zee HH, *et al*. Apremilast for moderate hidradenitis suppurativa: results of a randomized controlled trial. *J Am Acad Dermatol* 2019; 80(1): 80–88.

104. Kanni T, Zenker O, Habel M, *et al*. Complement activation in hidradenitis suppurativa: a new pathway of pathogenesis? *Br J Dermatol* 2018; 179: 413–419.

105. Kanni T, Argyropoulou M, Spyridopoulos T, *et al*. MABp1 Targeting IL-1alpha for moderate to severe hidradenitis suppurativa not eligible for adalimumab: a randomized study. *J Invest Dermatol* 2018; 138: 795–801.

106. Leung YY, Hui LLY and Kraus VB. Colchicine: update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum* 2015; 45: 341–350.

107. Armyra K, Kouris A, Markantoni V, *et al*. Hidradenitis suppurativa treated with tetracycline in combination with colchicine: a prospective series of 20 patients. *Int J Dermatol* 2017; 56: 346–350.

108. Jemec GB. Methotrexate is of limited value in the treatment of hidradenitis suppurativa. *Clin Exp Derm* 2002; 27: 528–529.

109. Heiberg MS, Rodevand E, Mikkelsen K, *et al*. Adalimumab and methotrexate is more effective than adalimumab alone in patients with established rheumatoid arthritis: results from a 6-month longitudinal observational multicenter study. *Ann Rheum Dis* 2006; 65: 1379–1383.

110. Cribbs AP, Kennedy A, Penn H, *et al*. Methotrexate restores regulatory T-cells function through demethylation of FOXP3

upstream enhancer in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2015; 67: 1182–1192.

111. Bianchi L, Hansel K and Stingeni L. Recalcitrant severe hidradenitits suppurativa successfully treated with cyclosporine A. *J Am Acad Dermtol* 2012; 67: 278–279.

112. Andreson MD, Zauli S, Bettoli V, *et al.* Cyclosporine treatment of severe hidradenitis suppurativa: a case series. *J Dermatol Treat* 2016; 27: 247–250.

113. Fonseca ACRG, Carvalho E, Eriksson JW, *et al.* Calcineurin is an important factor involved in glucose uptake in human adipocytes. *Mol Cell Biochem* 2018; 445: 157–168.

114. Blok JL, van Hattem S, Jonkman MF, *et al.* Systemic therapy with immunosuppressive agents and retinoids in hidradenitis suppurativa: a systematic review. *Br J Dermatol* 2013; 168: 243–252.

115. Tan MG, Shear NH, Walsh S, *et al.* Acitretin: monotherapy or combined therapy for hidradenitis suppurativa? *J Cut Med Surg* 2017; 21: 48–53.

116. Matusiak L, Biniek A and Szepietowski JC. Acitretin treatment for hidradenitis suppurativa: a prospective series of 17 patients. *Be J Dermtol* 2014; 171: 170–174.

117. Huang CM and Kirchhof MG. A new perspective on isotretinoin treatment of hidradenitis suppurativa: a retrospective chart review of patient outcomes. *Dermatology* 2017; 233: 120–125.

118. Raverdeau M and Mills KH. Modulation of T-cell and innate immune responses by retinoic acid. *J Immunol* 2014; 192: 2953–2958.

119. Yazdanyar S, Boer J, Ingvarsson G, *et al.* Dapsone therapy for hidradenitis suppurativa: a series of 24 patients. *Dermatology* 2011; 222: 342–346.

120. Wozel G and Blasum C. Dapsone in dermatology and beyond. *Arch Dermatol Res* 2014; 306: 103–124.

121. Schafer P. Apremilast mechanism of action and application to psoriasis and psoriatic arthritis. *Biochem Pharmacol* 2012; 83: 1583–1590.

122. Li H, Zuo J and Tang W. Phosphodiesterase-4 inhibitors for the treatment of inflammatory bowel diseases. *Front Pharmacol* 2018; 9: 1048.

123. Poveda I, Vilarrasa E, Martorell A, *et al.* Serum zinc levels in hidradenitis suppurativa: a case control study. *Am J Clin Dermatol* 2018; 19: 771–777.

124. Donnarumma M, Marasca C, Palma M, *et al.* An oral supplementation based on myo-inositol, folic acid and liposomal magnesium may act synergistically with antibiotic therapy and can improve metabolic profile in patients affected by hidradenitis suppurativa: our experience. *G Ital Dermatol Venereol.* Epub ahead of print 20 September 2018. DOI: 10.23736/S0392-0488.18.06012-1.

125. Igic PG, Lee E, Harper W, *et al.* Toxic effects associated with consumption of zinc. *Mayo Clin Proc* 2002; 77: 713–716.

126. Baker KF and Isaacs JD. Novel therapies for immune-mediated inflammatory diseases: what can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis? *Ann Rheum Dis* 2018; 77: 175–187.

127. Frew JW, Vekic DA, Woods J, *et al.* Phenotypic heterogeneity implies heterogeneous pathogenic pathways in hidradenitis suppurativa. *Exp Derm* 2015; 24: 338–339.

128. Hawkes JE, Yan BY, Chan TC, *et al.* Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. *J Immunol* 2018; 201: 1605–1613.

129. Cotter C, Tobin AM, O'Connor R, *et al.* Severe refractory hidradenitis suppurativa: treatment with ixekizumab, two case reports. *Br J Dermatol* 2018; 179: 70.

130. Pandey K, Sekhri R and Sekhri V. Interleukin-17 inhibitor secukinumab in refractory hidradenitis suppurativa: a case report. *J Am Acad Dermatol* 2018; 79: AB156.

131. Principi M, Cassano N, Contaldo A, *et al.* Hidradentiis suppurativa and inflammatory bowel disease: an unusual but existing association. *World J Gastroenterol* 2016; 22: 4802–4811.

132. Hueber W, Sands BE, Lewitzky S, *et al.* Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut* 2012; 61: 1693–1700.

133. Tegla CA, Cudrici C, Patel S, *et al.* Membrane attack by complement: the assembly and biology

of terminal complement complexes. *Immunol Res* 2011; 51: 45–60.

134. Schmudde I, Ströver HA, Vollbrandt T, *et al*. C5a receptor signaling in dendritic cells controls the development of maladaptive Th2 and Th17 immunity in experimental allergic asthma. *Mucosal Immunol* 2013; 6: 807–825.

135. Jensen L. Targeting the IL-1 family in skin inflammation. *Curr Opin Invest Drugs* 2010; 11: 1211–1220.

136. Collantes-Rodriguez C, Ossorio-Garcia L, Villegas-Romero I, *et al*. A single case of hidradenitis suppurativa, Crohn's disease, and psoriasis treated with ustekinumab. *J Am Acad Dermatol* 2018; 79: AB24.

137. Blok JL, Li K, Brodmerkel C, *et al*. Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. *Br J Dermatol* 2016; 174: 839–846.

138. Low CM, Akthar S, Patel DF, *et al*. The development of novel LTA14 modulators to selectively target LTB4 generation. *Sci Rep* 2017; 7: 44449.

139. Strober BE, Kim C and Siu K. Efalizumab for the treatment of refractory hidradenitis suppurativa. *J Am Acad Dermatol* 2007; 57: 1090–1091.

140. Cavet J, Dickinson AM, Norden J, *et al*. Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. *Blood* 2001; 98: 1594–1600.

141. Welsch K, Holstein J, Laurence A, *et al*. Targeting JAK/STAT signaling in inflammatory skin diseases with small molecule inhibitors. *Eur J Immunol* 2017; 47: 1096–1107.

142. Torma H. Regulation of keratin expression by retinoids. *Dermatoendocrinol* 2011; 3: 136–140.

143. van de Kerkhof PC. Update on retinoid therapy of psoriasis in: an update on the use of retinoids in dermatology. *Dermatol Ther* 2006; 19: 252–263.

4.2: Integrating New Knowledge into the existing pathogenic paradigm of HS:

Manuscript	Manuscript Reference
2-1	Frew JW, Navrazhina K, Maroun M, Lu PJ, Krueger JG Contribution of Fibroblasts to Tunnel Formation and Inflammation in Hidradenitis Suppurativa Exp Dermatol 2019 28(8):886-891
2-2	Grand D, Navrazhina K, Frew JW Integrating Complement into the Molecular Pathogenesis of Hidradenitis Suppurativa Exp Dermatol 2020; 29(1): 86-92
2-3	Frew JW, Grand D, Navrazhina K, Krueger JG "Beyond Antibodies: B-cells in Hidradenitis Suppurativa: Bystanders, Contributors or Therapeutic Targets?" Exp Dermatol 2020; 29(5):509-515
2-4	Frew JW. Hidradenitis Suppurativa is an Autoinflammatory Keratinization Disease: A Review of the Clinical, Histological and Molecular Evidence: JAAD International 2020;1(1):62-72
2-S1	Navrazhina K, Frew JW, Garcet S, Krueger JG. Epithelialized Tunnels Contribute to Inflammation in Hidradenitis Suppurativa. J Allergy Clin Immunol 2020; doi:10.1016/j.jaci.2020.12.651
2-S2	Navrazhina K, Garcet S, Gonzales J, Grand D, Frew JW, Krueger JG In-Depth Analysis of the Hidradenitis Suppurativa Serum Proteome Identified Distinct Inflammatory Subtypes. J Invest Dermatol 2021; doi:10.1016/j.jid.2021.02.742

Table 4-2-1 Publications Presented in this chapter

The publications contributing to the first chapter of this dissertation identified that various inflammatory cell types are present in the setting of disease activity in HS. However, novel testable hypotheses regarding their mechanism of action has largely been absent from the literature to date. A plethora of literature has investigated the complex role of the innate and adaptive immune system in various other inflammatory

skin diseases such as psoriasis vulgaris[63] and atopic dermatitis[64], including the role of specific cell subsets such as fibroblasts and B-cells[63,64]. In contrast, relatively little has been published regarding the complexities of the innate and adaptive immune system in HS.

The first 3 publications contributing to this chapter (2-1, 2-2, 2-3) critically examine the role of fibroblasts, complement and B-cells, and by using knowledge from other inflammatory skin disorders and other related disorders (including inflammatory bowel disease and pyoderma gangrenousm), develop testable hypotheses regarding their potential roles in the pathogenesis of HS. These novel discussions of the complex inflammatory milieu identified in HS is a significant departure from the previous paradigm in which HS is characterised primarily by the superficial dermal and epidermal changes in the disease. Certainly, the findings form the systematic reviews presented in chapter 1 identify the types of superficial biopsies taken in many observational studies and the unconscious cognitive bias towards primarily epidermal disorders (Psoriasis and atopic dermatitis) may play some role in the lack of investigations into the deep dermal component of disease.

The fourth publication contributing to this chapter (2-4) is an overarching analysis of how novel insights into the inflammatory nature of the disease, the influence of morphological heterogeneity upon molecular markers of systemic inflammation and the efficacy of various therapeutics is shifting the pathogenic paradigm of HS. The concept of ‘follicular occlusion’ as the *primem movens* of HS has not been challenged despite multiple publications suggesting the occlusion and inflammation are at a minimum co-existant[65,66]. Numerous inconsistencies regarding the precise mechanisms of follicular

rupture[67] and how this is different from other disorders which demonstrate follicular occlusion (acne vulgaris, keratosis pilaris etc) have not been addressed in the setting of the follicular occlusion paradigm. An alternative hypothesis which correlates with the existing molecular and clinical observations is HS being an autoinflammatory keratinisation disorder with follicular occlusion being a secondary phenomenon mediated by IL-1α.

The two supplementary publications relevant to this chapter (2-S1, 2-S2) present novel data taken from observational studies examining the tissue and serum inflammatory profiles of patients with untreated HS. The first supplementary publication (2-S1) pertains to the immunological structure and function of epithelialised tunnels (previously known as sinus tracts or fistulae). This publication demonstrates that they reliably recapitulate the structure of the overlying epidermis (including cell types such as melanocytes, Langerhans cells etc) and demonstrates that they have a greater relative contribution to tissue inflammation than the overlying epidermis. These novel structures are not seen in any other skin disease and their inflammatory function emphasises the concept that specific targeted therapy is needed to address the role of epithelialised tunnels in the disease.

The second supplementary publication (2-S2) presents novel data regarding the heterogeneity of systemic inflammation via serum proteomics in untreated HS. Whilst correlation with disease severity has been demonstrated in other studies[20], the link between systemic inflammation and the presence of draining epithelialised tunnels is a novel observation and the major finding in this publication.

4.2.1: Publication 2-1

Frew JW, Navrazhina K, Maroun M, Lu PJ, Krueger JG Contribution of Fibroblasts to Tunnel Formation and Inflammation in Hidradenitis Suppurativa Exp Dermatol 2019 28(8):886-891

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4.2.2: Publication 2-2

Grand D, Navrazhina K, Frew JW Integrating Complement into the Molecular Pathogenesis of Hidradenitis Suppurativa Exp Dermatol 2020; 29(1): 86-92

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https://onlinelibrary.wiley.com/doi/10.1111/exd.14056

4.2.3: Publication 2-3

Frew JW, Grand D, Navrazhina K, Krueger JG "Beyond Antibodies: B-cells in Hidradenitis Suppurativa: Bystanders, Contributors or Therapeutic Targets?" Exp Dermatol 2020; 29(5):509-515

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https://onlinelibrary.wiley.com/doi/10.1111/exd.14092

4.2.4: Publication 2-4

Frew JW. Hidradenitis Suppurativa is an Autoinflammatory Keratinization Disease: A Review of the Clinical, Histological and Molecular Evidence: JAAD International 2020;1(1):62-72

Hidradenitis suppurativa is an autoinflammatory keratinization disease: A review of the clinical, histologic, and molecular evidence

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The pathogenic model of hidradenitis suppurativa is in the midst of a paradigm shift away from a disorder of primary follicular occlusion to an autoinflammatory keratinization disease. Observational, experimental, and therapeutic evidence supports the concept of hidradenitis suppurativa as a primarily inflammatory disorder, a disorder of autoimmunity, or both, in contrast to the current prevailing paradigm of primary follicular occlusion. The lack of reliable and high-fidelity disease models has limited the available experimental and mechanistic evidence to support or refute one pathogenic model over another. This scholarly review synthesizes the existing clinical, histologic, and molecular data to evaluate the extant evidence supporting the autoinflammatory paradigm and further informing the molecular mechanisms of hidradenitis suppurativa pathogenesis. Follicular hyperkeratosis/occlusion and perifollicular inflammation coexist in histologic specimens, with interleukin 1α demonstrated to stimulate comedogenesis in the infundibulum. pH elevation in occluded body sites alters the microbiome and amplifies existing T-helper cell type 17 immunoresponses. Known metabolic comorbidities and smoking are known to upregulate interleukin 1α in follicular keratinocytes. Identified genetic variants may alter epidermal growth factor receptor signaling, leading to upregulated keratinocyte inflammatory responses. The process of follicular rupture and dermal tunnel formation can be explained as secondary responses to inflammatory activation of fibroblasts and epithelial-mesenchymal transition, with antibody production associated with inflammatory amplification in advanced disease. This review aims to reevaluate and integrate the current clinical, histologic, and molecular data into a pathogenic model of hidradenitis suppurativa. This is essential to advance our understanding of the disease and identify novel therapeutic targets and approaches. (JAAD Int 2020;1:62-72.)

Key words: acne inversa; autoinflammatory; hidradenitis suppurative; inflammation; mechanism; pathogenesis.

INTRODUCTION

The pathogenic model of hidradenitis suppurativa is in the midst of a paradigm shift[1] away from a disorder of (primary) follicular occlusion[2] to an autoinflammatory keratinization disease.[1] There is observational, experimental, and therapeutic evidence to support the concept of hidradenitis suppurativa as a primarily inflammatory disorder,[1] a disorder of autoimmunity[3] (in contrast to that primarily of follicular occlusion), or both (Fig 1); however, the lack of reliable disease models[4,5] has limited experimental and mechanistic evidence to support or refute one pathogenic model over another (Fig 1). This review aims to reevaluate and integrate the current clinical, histologic, and molecular data into a pathogenic model of hidradenitis suppurativa. This is essential to advance our understanding of the disease[6] and identify novel therapeutic targets.[7,8]

FOLLICULAR OCCLUSION IS CLINICALLY

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AND EXPERIMENTALLY A PRODUCT OF INFLAMMATION RATHER THAN A CAUSE

Follicular hyperkeratosis and comedogenesis coexist with perifollicular inflammation in hidradenitis suppurativa.[9-11] Comedones are present in flexural and nonflexural inflamed and scarred tissues, as well as noninflamed tissues.[9-11] Subclinical inflammation (observed in hidradenitis suppurativa)[12,13] precedes comedogenesis in acne-prone skin,[14-20] involving keratinocyte-derived proinflammatory mediators (lipoteichoic acid, CCL20, and interleukin [IL] 1α).[18-24] Ex vivo studies of the follicular infundibum[25] isolated in vitro are able to recapitulate the formation of comedones with addition of IL-1α and prevent formation with the addition of IL-1 receptor antagonist.[25] It is acknowledged that the in vitro studies performed are based on highly sebaceous follicular units that have distinct differences from apocrine-bearing skin[26]; however, the similarities in immunologic milieu between sebaceous and apocrine skin in T-helper cell 17–associated mediators[7,26] (central to inflammation in hidradenitis suppurativa)[26] raises the possibility that these mechanisms are shared. Molecular and ex vivo evidence suggests comedo formation may be secondary to subclinical inflammation. These results may explain the diffuse scattering of comedones observed in hidradenitis suppurativa–prone areas, the presence of comedones in extraflexural sites, and their presence in previously inflamed ("burned-out") tissue or sites distant from a follicular unit.[9,10]

CAPSULE SUMMARY

- Hidradenitis suppurativa is known as a disorder of follicular occlusion, with inflammation being a primary manifestation of disease. Clinical, histologic, and molecular evidence suggests that inflammation is central to multiple aspects of disease, but this remains poorly integrated into the existing pathogenic paradigm.
- Histologic and molecular evidence supports the concept of inflammation as the primary driver of disease activity in hidradenitis suppurativa. It enables explanation of observed events such as follicular rupture, tunnel formation, and systemic inflammation, which are poorly described in the follicular occlusion paradigm. Hidradenitis suppurativa is an autoinflammatory keratinization disease. Reframing our pathologic and clinical understanding in the context of this paradigm is vital to identify and implement novel therapeutic strategies for this burdensome disease.

SKIN FOLD OCCLUSION IS ASSOCIATED WITH MICROBIOME ALTERATIONS AND SUBSEQUENT PROINFLAMMATORY KERATINOCYTE RESPONSES

From a clinical perspective, follicular occlusion may refer to anatomic sites of disease predilection (axillary, inguinal, and submammary folds).[2] These areas demonstrate alterations in moisture, pH, and microbiological colonization[2] (Fig 2), particularly in the setting of obesity.[27-29] The follicular infundibulum is an immunologically active, microbially colonized site[23,30,31] involved in the development of immune tolerance to commensal organisms.[23,30,31] This differs substantially from other portions of the follicle (such as the bulb), which are considered immunologically privileged sites.[32] Infundibular keratinocytes produce CCL20 and antimicrobial peptides under normal physiologic conditions[23] (Fig 2). Increasing moisture decreases the pH of the stratum corneum,[28,29] promoting the colonization and activity of hidradenitis suppurativa–associated microbionts (eg, *Porphyromonas*)[33,34] (Fig 2). Other bacteria,[35,36] yeasts,[37] and associated proteins (including lipoteichoic acid) induce the release of preformed IL-1α in keratinocytes.[38] Indirect evidence for the role of yeasts in inflammatory activity in hidradenitis suppurativa[39] has been demonstrated in recent observational studies.[39,40] Although the precise mechanisms of specific microbiological species and strains in hidradenitis suppurativa is ill defined, their functional role in producing an aberrant proinflammatory response (either directly or indirectly via keratinocytes) is consistent with observational studies identifying these microbionts in both early and advanced disease.[34,35]

INFLAMMATION IN HIDRADENITIS SUPPURATIVA: EVIDENCE FROM EXISTING STUDIES

The inflammatory signature of established hidradenitis suppurativa has been well characterized in multiple histologic[26,41] and molecular studies.[26,42-45] Similarities and parallels with psoriasis[26] have been observed in lesional and perilesional hidradenitis suppurativa tissue,[26,46] with lesional nodules demonstrating mixed inflammatory infiltrates comprising T cells, dendritic cells, plasma cells, neutrophils,[47-50] and monocytes.[51] Chronic long-

Abbreviations used:

EGFR: Epidermal growth factor receptor
IL: interleukin

standing disease appears autoinflammatory[52-54] and also demonstrates B-cell infiltrates,[3,12] NETosis,[3] and development of epithelialized tunnels.[55] An issue with understanding the characteristics of inflammation in hidradenitis suppurativa is that the majority of specimens isolated for studies are from individuals with severe, long-standing disease.[3,12,13] Hence, we have limited insight into the initiating events in early and mild hidradenitis suppurativa. Additionally, until recently there were no standardized, defined biopsy sites for investigational studies.[56] Given that hidradenitis suppurativa is morphologically diverse, it would be erroneous to assume that a biopsy from one portion of tissue is representative of all the different epidermal (and deep dermal) morphologies present across the spectrum of hidradenitis suppurativa.[56] Therefore, studies that do not define the severity, treatments, sites, and lesion types of biopsies should be interpreted with caution.[41,42]

The mechanisms of lesion development are unclear because perilesional inflammation is of the same character (albeit less intense) as nearby lesional inflammation[42,43,57]; however, lesional cytokine profiles are unable to be experimentally generated from the addition of IL-1α, IL-1β, or both to perilesional tissue.[5,57] This raises the prospect that the process of inflammation in hidradenitis suppurativa is more complex than initially thought and that the inflammatory characteristics of perilesional tissue are distinct from those of lesional tissue.[5]

DISEASE INITIATION IS ASSOCIATED WITH SYSTEMIC SUBCLINICAL INFLAMMATION AND DYSREGULATED INFUNDIBULAR KERATINOCYTES

Understanding of the initiating factors associated with the excessive and self-perpetuating perifollicular inflammation in hidradenitis suppurativa remains incomplete. Epidemiologic and clinical observations suggest that a number of systemic disorders (including insulin resistance, hormonal dysregulation, and obesity) may be associated with hidradenitis suppurativa[58] and contribute to a proinflammatory state[59,60] (Fig 2). In other inflammatory disorders, such as psoriasis,[61] rheumatoid arthritis,[62] and atherosclerosis,[63] these factors have been found to be associated. However, the causation between disease and systemic inflammation is still a topic of contention.[64]

Guidelines[65,66] and clinical evidence[67,68] suggest weight loss, smoking cessation, and dietary counseling as an integral part of hidradenitis suppurativa management[65,66] through suppression of inflammation.[69-71] Smoking, via polycyclic aromatic hydrocarbons, can directly alter follicular keratinocyte differentiation, resulting in comedogenesis.[72] It can also produce widespread methylation changes and systemic increases in IL-6, C-reactive protein, fibrinogen, and multiple members of the nuclear factor kappa-light-chain-enhancer of activated B cells family.[71] Adipose tissue can produce proinflammatory signatures, including IL-6, IL-1β, and tumor necrosis factor-α in the setting of chronic nutrient excess.[69,70] Additionally, adipokines can mediate both inflammation and the development of insulin resistance[73] (Fig 2), which is also associated with hidradenitis suppurativa.[58] Keratinocytes in the infra-infundibulum of the follicle express type 1 5-hydroxytestosterone,[74] modulating infundibular keratinocyte differentiation programs both directly[75] and via fibroblast activation and fibroblast-keratinocyte interactions, contributing to androgen-induced follicular changes.[74]

Overall, these associations suggest that a systemic proinflammatory state and localized infundibular keratinocyte dysregulation are potential predisposing factors to clinical disease. There are contradictory reports[76] pertaining to the benefit of withdrawing these predisposing factors (eg, cessation of smoking, weight loss) during established disease. These findings appear contradictory only if one holds the assumption that the initiating and perpetuating factors of clinical disease in hidradenitis suppurativa are one and the same. As other authors have suggested,[77] there may be unique factors contributing to each state (initiation of disease and perpetuation of disease); and our lack of data regarding early (subclinical) disease has not allowed us to appreciate this fact.[77]

T-HELPER CELL 17 FEED-FORWARD INFLAMMATION IS PROMINENT IN ESTABLISHED DISEASE

The T-helper cell 17 axis is strongly implicated in established self-perpetuating clinical disease[26]; however, the mechanisms leading to T-helper cell 17 feed-forward self-amplification in hidradenitis suppurativa are still unclear. It is assumed to be similar to the activation of the T-helper cell 17 axis in psoriasis,[78] with the predisposition of the axillae and other areas of apocrine-gland-rich skin to a T-helper cell 17 immunoresponse, as demonstrated

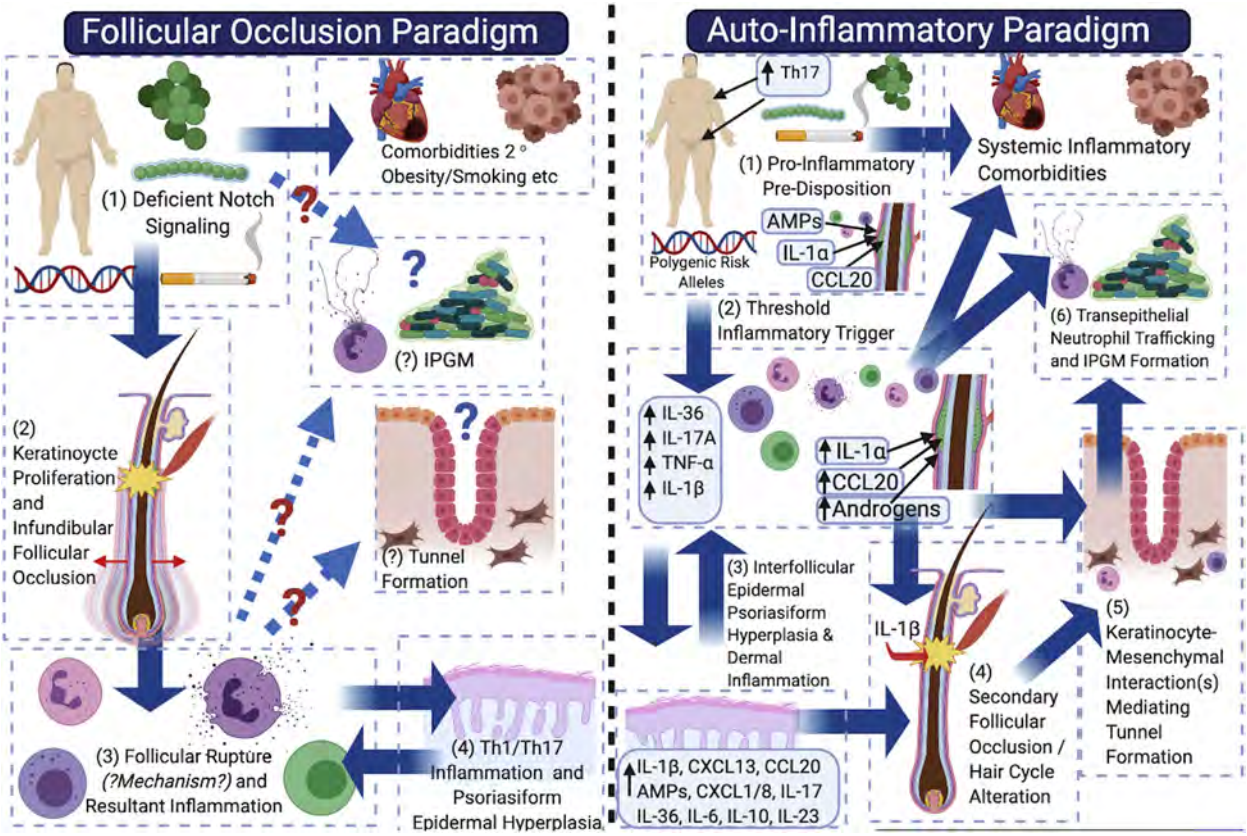

Fig 1. Schematic representation and comparison of the follicular occlusion paradigm (left panel) and the autoinflammatory paradigm (right panel) in the pathogenesis of hidradenitis suppurativa. In the follicular occlusion paradigm, deficient Notch signaling (1) directly results in infundibular keratinocyte proliferation and follicular occlusion (2), leading to follicular dilatation, rupture, and resultant inflammation. One deficiency of this paradigm is the lack of hypothesized mechanisms by which rupture occurs and why deep follicular rupture occurs preferentially to expulsion of the comedo. The resultant T-helper cell 1/17 inflammatory axis (3) (4) then results in the observed inflammatory profile of disease; however, no clear mechanism is hypothesized for how tunnels form and how the infiltrative proliferative gelatinous mass results. The autoinflammatory paradigm (right panel) places inflammation as the primary driver of disease, with subclinical inflammation (1) developing as a result of disparate contributing factors on a background of topographic predisposition. Dermal inflammatory infiltrates (2) then drive secondary follicular occlusion (3 and 4), with resultant tunnel formation a consequence of keratinocyte-mesenchymal interactions (5) that mimic outer-root sheath keratinocyte downgrowth in follicular development in early anagen. Chemokine gradients in epithelialized tunnels then drive neutrophil trafficking to the lumen and formation of the infiltrative proliferative gelatinous mass (6).

experimentally.[25] There is well-documented evidence (largely from the psoriasis literature) regarding feed-forward mechanisms between IL-1β, IL-6, and tumor necrosis factor-α by IL-17,[78,79] leading to further IL-1β, IL-6, and tumor necrosis factor-α production, as well as downstream activation of acute phase reactants and neutrophilic and complement-mediated inflammatory responses.[78-80] This is further perpetuated through leucocyte-keratinocyte interactions,[78-80] further amplifying antimicrobial peptide and chemokine production (including CXCL1 and CXCL8),[81] leading to further inflammatory cell recruitment adjacent to IL-17–activated epidermal keratinocytes (Fig 3). Such inflammatory cell localization has been observed surrounding intrafollicular and interfollicular sites adjacent to epidermal keratinocytes in early histologic specimens of hidradenitis suppurativa,[9-11,64] with evidence of early psoriasiform hyperplasia suggestive of IL-17–induced epidermal changes. Despite that the majority of translational work focuses on IL-17A (given the body of preexisting

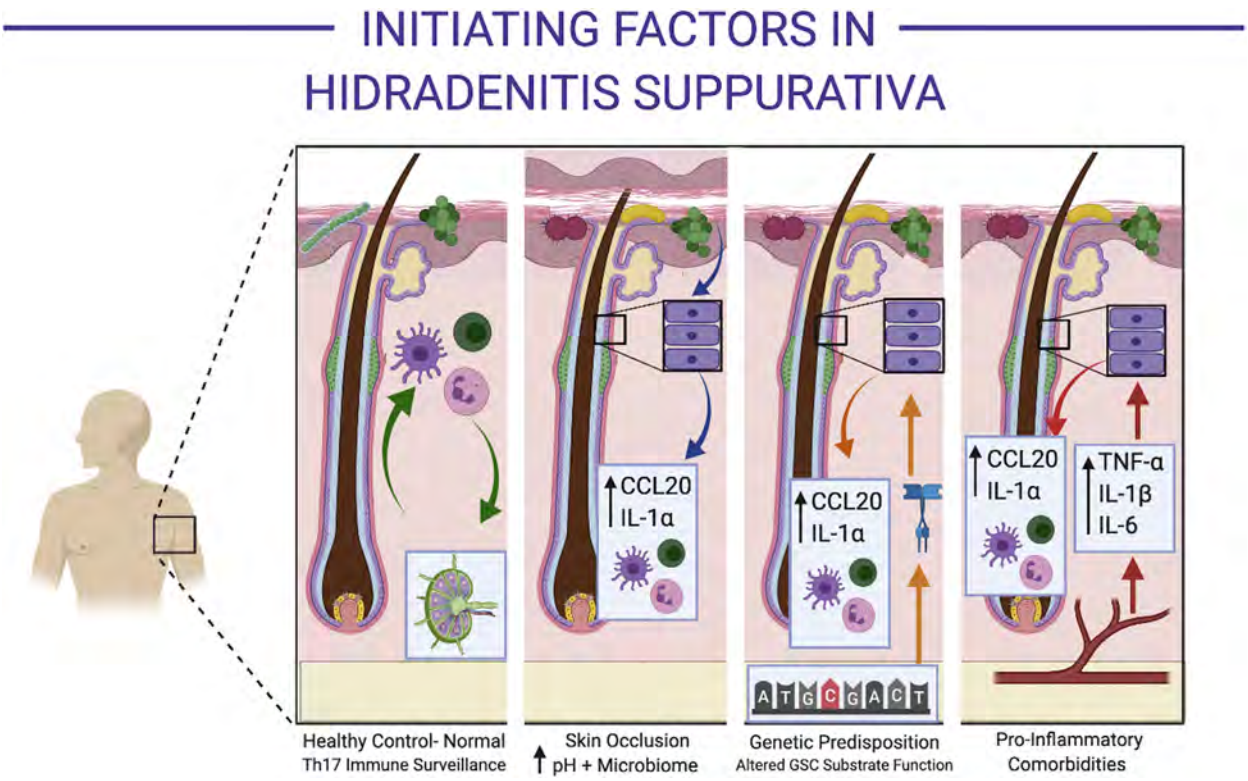

Fig 2. Initiating factors in hidradenitis suppurativa. Normal control skin (first panel from the left) from hidradenitis suppurativa–associated cutaneous sites (eg, axilla) have normal colonizing microbionts (including within the follicular infundibulum), which are continuously monitored by circulating immune cells in homeostasis (circulating to and from regional lymph nodes (inset in first panel from the left). Known predisposing factors, including skin occlusion (second panel from left), predisposing genetic mutations (third panel from the left), and proinflammatory comorbidities such as obesity and insulin resistance, increase the inflammatory drive of infundibular keratinocytes (purple rectangular cells) via varied mechanisms. Skin occlusion (second panel from the left) alters the microbiological composition of the skin (red and yellow microbionts) via increases in cutaneous pH. These microbionts increase the production of CCL20 and interleukin (IL) 1α by infundibular keratinocytes. Genetic mutations in the γ-secretase complex are known to affect Notch signaling and also substrates including epidermal growth factor receptors, which are active in the follicular infundibulum. Dysregulation of EGFR signaling is known to increase CCL20 and IL-1α production by infundibular keratinocytes. Metabolic comorbidities produce increased levels of circulating tumor necrosis factor-α, IL-1β, and IL-6. These mediators stimulate CCL20 and IL-1α production.

work based on psoriasis), significant elevations of other IL-17 isoforms, including IL-17C and IL-17F, are observed in hidradenitis suppurativa tissue,[81,82] and these may be significant contributors to disease activity that are not targeted by anti-IL-17A therapies alone.

THE ROLE OF B CELLS, DESPITE THEIR DOMINANCE, REMAINS UNCLEAR

Long-standing and severe disease may have a unique inflammatory profile compared with milder or less established forms of hidradenitis suppurativa. Histologic and transcriptomic studies[44,45] have identified a high level of B-cell[3] and plasma-cell[12,13] signatures, complement (specifically C5a) activation,[47-50] and extensive tissue remodeling via matrix metalloproteinases with subsequent destruction of follicular and glandular structures in the dermis.[2,11] The role and characteristics of B cells in mild to moderate hidradenitis suppurativa are unclear.[83] The presence of B cells and plasma cells in skin and blood[3,12,13] suggests the possibility that some component of severe or long-standing hidradenitis suppurativa may be an autoimmune or antibody-mediated disorder. However, to date no product has been definitively identified as an autoimmune target for the disease.[83] B cells are present in other chronic inflammatory disorders without known

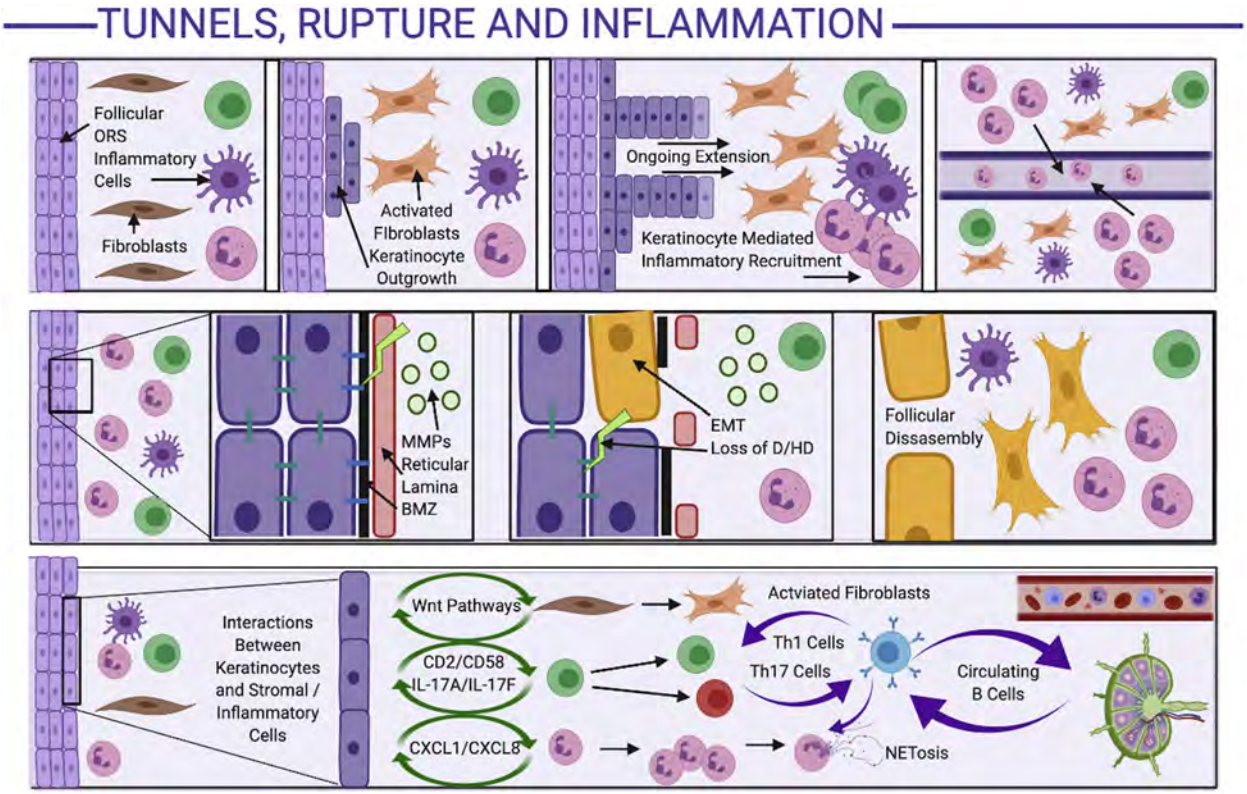

Fig 3. Mechanisms of tunnel formation, follicular rupture, and perpetuation of inflammation in hidradenitis suppurativa. Development of tunnels (top panel): Inflammation adjacent to the follicular outer root sheath activates fibroblasts, with stromal-keratinocyte feedback resulting in keratinocyte outgrowth from the follicular wall. The ongoing keratinocyte outgrowth results in keratinocyte-mediated inflammatory cell recruitment, further amplifying the stromally mediated keratinocyte outgrowth in a positive-feedback loop. The inflammatory cells are attracted to the keratinocyte chemokine (CXCL1/CXCL8) gradient, resulting in migration into the lumen of the tunnels. Mechanisms of follicular rupture (middle panel): The inflammatory infiltrate is associated with high levels of matrix metalloproteinases, which degrade the reticular lamina. Keratinocyte-leucocyte cross talk activated epithelial-mesenchyme-transition mechanisms, leading to degradation of the basement membrane zone, loss of hemidesmosomes and desmosomes, and keratinocytes expressing mesenchymal cell surface markers (yellow keratinocytes). Eventually, the follicular wall is disassembled, replaced by mesenchymal cells and dense inflammatory infiltrates. Mechanisms of inflammatory amplification (bottom panel): Activated keratinocytes interact with inflammatory and stromal cells via various pathways to result in activated fibroblasts, T-helper cell types 1 and 17, and infiltration of dendritic cells and neutrophils. Circulating B cells (circulating to and from regional lymph nodes; far right of lower panel), activated by the high-interferon-mediated milieu, interact with multiple cell types to amplify existing inflammatory loops, as well as recirculate in the lymphatic and vascular system, contributing to systemic inflammation. *D*, Desmosome; *HD*, hemidesmosome; *MMP*, matrix metalloproteinase; *ORS*, outer root sheath.

autoimmune targets, including psoriasis and atopic dermatitis.[84] In these conditions, they are thought to be bystanders (secondary to combined B-cell and T-cell chemoattractants such as CXCL13 or CCL20) or secondary amplifiers of T-cell–mediated inflammation.[83] (Fig 3). Byrd et al[3] demonstrated that antibodies to citrullinated peptides contribute to the development of neutrophil extracellular traps in advanced disease, with parallels to B-cell and neutrophil extracellular traps in rheumatoid arthritis.[3] Case reports of rituximab ameliorating hidradenitis suppurativa disease activity are known,[83] but overall, the role of B cells as bystanders, amplifiers of existing inflammation, or central pathogenic players is unclear and requires further investigation.[83]

GENETIC VARIANTS IN HIDRADENITIS SUPPURATIVA MAY ACT VIA EGFR-ASSOCIATED PATHWAYS LINKING FOLLICLES, T-HELPER CELL 17–MEDIATED INFLAMMATION, AND DRUG-INDUCED DISEASE

A minority of patients with familial and spontaneous hidradenitis suppurativa have been identified with *GSC* mutations.[85] The precise mechanism of action of *GSC* mutations in the pathogenesis of hidradenitis suppurativa is unclear.[86] The *GSC* complex cleaves more than 70 different substrates involved in cell cycle and inflammation, including epidermal growth factor receptor (EGFR), IL-1, tumor necrosis factor-α, and Notch.[86] Notch is proposed as the unifying motif in hidradenitis suppurativa pathogenesis via associations with keratinocyte proliferation,[87] smoking, and sequence variants in *GSC*.[88,89] However, Notch dysregulation is also present in multiple other inflammatory dermatoses,[90] arguing against a unique role in hidradenitis suppurativa. *In silico* evidence[86] has identified *ERbb4* and *Tie1* as differentially expressed *GSC* substrates that distinguish the transcriptome of hidradenitis suppurativa from familial Alzheimer disease and other inflammatory skin diseases.[90] These components of the EGFR pathway (active in the follicular infundibulum[23]) are associated with *SOX9* and *Wnt* signaling linked with hair cycle progression, IL-17A production[23,91] (through shared downstream Act1 activity), and epithelial cell fate,[91] all mechanisms identified in transcriptomic analysis of hidradenitis suppurativa tissues.[44,77] *GSC* knockdown results in IL-36α production,[92] alterations in EGFR signaling,[93] and increased sensitivity to interferon-mediated proinflammatory pathways[92] (Fig 2). POFUT-1 (identified in cases of Dowling-Degos disease associated with hidradenitis suppurativa[94,95]) is a fucosyltransferase that is active on multiple substrates, including Notch and EGFR,[96] and is important for posttranslational modification of receptors.[96] This suggests a role for EGFR signaling in hidradenitis suppurativa, supported by reports of hidradenitis suppurativa associated with use of EGFR antagonists in oncology.[97]

THE EVIDENCE AND PROPOSED MECHANISMS FOR FOLLICULAR RUPTURE

Follicular rupture is proposed as the primary mechanism by which follicular occlusion leads to dermal inflammation in hidradenitis suppurativa, but the molecular mechanisms remain unclear.[2] Observational studies demonstrate the coexistence of dense perifollicular and intrafollicular inflammation and discontinuities in follicular epithelium in affected tissues[9,10,64] (Fig 3). Long-standing disease demonstrates a noticeable absence of follicular and adnexal structures,[98] consistent with profound dermal inflammation. A reduction in the thickness of the fibroreticular lamina surrounding follicles and sebaceous glands[99] has been observed. Occluded follicles in other conditions (such as epidermal inclusion cysts[100]) are testament to the potential size intrafollicular collections may progress to before rupture. However, the early presence of inflammation in hidradenitis suppurativa lesions suggests an inflammation-related mechanism[101] that is well documented to disassemble the basement membrane zone as part of the wound-healing process.[102] Epithelial-mesenchymal transition pathways[103] are part of the normal wound-healing response and have been identified in transcriptomic analysis of hidradenitis suppurativa tissues.[84,104] It may also explain the presence of keratin-staining cells in the dermis of hidradenitis suppurativa sections[41] (via keratinocytes undergoing epithelial-mesenchymal transition but still expressing keratin proteins), the destruction of follicular and adnexal structures in advanced disease,[98] and the development of dermal tunnels[103] (Fig 3). Similar inductions in epithelial-mesenchymal transition-associated signaling pathways are observed in malignancy and wound healing and contribute to the metastatic potential of cancer and long-standing wounds.[105] Hence, the concept of follicular rupture may be more appropriately described as a process of "follicular disassembly" (Fig 3) induced by the chronic inflammatory changes via epithelial-mesenchymal transition and aberrant extracellular remodeling wound-healing programs.[103]

DERMAL TUNNELS ARE ACTIVE INFLAMMATORY STRUCTURES AND THEIR DEVELOPMENT IS ORCHESTRATED BY DERMAL INFLAMMATION

Dermal tunnels in hidradenitis suppurativa are unique structures comprising stratified squamous epithelia that recapitulate the structure of the overlying epidermis and produce active inflammatory mediators.[106] This is in contrast to other tunnel-like structures in chronic inflammatory conditions such as fistulizing Crohn's disease, which do not recapitulate mucosal structures with the same degree of fidelity.[107] The mechanisms leading to tunnel formation are unclear; however, it is hypothesized that these tunnels derive from the aberrant keratinocyte outgrowth from the outer root sheath of the

follicle[103] (Fig 3). Tunnels do not extend into the subcutaneous tissues or fistulize with other hollow organs (except in the context of coexistent inflammatory bowel disease), suggesting an association with signaling from the dermis.[103] This parallels the development of the hair follicle and early anagen downgrowth in the hair cycle,[105,108] which are mediated via platelet derived growth factor α-derived signaling from the dermal condensate.[105] Platelet derived growth factor α-mediated signaling has also been identified in transcriptomic data from hidradenitis suppurativa–associated fibroblasts.[103] Given that these fibroblast-derived signals are secondary to inflammation-mediated epigenetic modifications,[103] it is plausible to assume that the development of tunnels is an inflammation-driven process. However, once these tunnels are established, the CXCL1/8 gradient established across the epithelia[106] (including tunnels) results in transepithelial neutrophil trafficking and neutrophil extracellular trap formation in tunnel lumen.[3] This results in development of the infiltrative proliferative gelatinous mass[109] and biofilm formation in hidradenitis suppurativa tunnels[110] (Fig 3). This in turn drives further inflammatory recruitment surrounding these established tunnels, leading to the ongoing cycle of severe intractable inflammation and drainage.

CONCLUSIONS

The available histologic and molecular evidence suggests inflammation is a central component to the pathogenesis of hidradenitis suppurativa. Placing inflammation as the primary driver of disease provides a scaffold for testable hypotheses regarding polygenic risk loci for the development of hidradenitis suppurativa, drug-induced causes of hidradenitis suppurativa, the development of dermal tunnels, and the inflammatory proliferative gelatinous mass, which are currently poorly integrated into the follicular occlusion model of hidradenitis suppurativa (Fig 1). More mechanistic and translational investigations are needed to further evaluate the role of genetics and B cells in hidradenitis suppurativa, as well as provide mechanistic evidence about the development of follicular rupture and tunnel formation. Such basic cellular and molecular investigations are vital to develop our understanding of the disease. Realigning the pathogenic paradigm with the molecular evidence is essential to enable the identification and exploration of novel targets, interventions, and therapeutics for this chronic debilitating disease.

REFERENCES

1. Akiyama M, Takeichi T, McGrath JA, Sugiura K. Autoinflammatory keratinization diseases. *J Allergy Clin Immunol*. 2017; 140:1545-1547.
2. Vossen ARJV, van der Zee HH, Prens EP. Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model. *Front Immunol*. 2018;9:2965.
3. Byrd AS, Carmona-Rivera C, O'Neil LJ, et al. Neutrophil extracellular traps, B cells, and type I interferons contribute to immune dysregulation in hidradenitis suppurativa. *Sci Transl Med*. 2019;11(508):eaav5908.
4. Van der zee HH, Laman JD. Prens EP. Can animal skin diseases or current transgenic mice serve as a model for hidradenitis suppurativa? *Dermatology*. 2012;225(1):9-13.
5. Frew JW, Piguet V. Ex-vivo models and interpretation of mechanistic studies in hidradenitis suppurativa. *J Invest Dermatol*. 2020;140(7):1323-1326.
6. Frew JW. Commentary: hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model. *Front Immunol*. 2019;10:302.
7. Czarnowicki T, He H, Krueger JG, Guttman-Yassky E. Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol*. 2019;143(1):1-11.
8. Frew JW, Marzano A, Wolk K, et al. Identifying novel therapeutic targets in hidradenitis suppurativa. *J Invest Dermatol*. 2020 (In Press).
9. Von Laffert M, Stadie V, Wohlrab J, Marsch WC. Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae. *Br J Dermatol*. 2011;164(2): 367-371.
10. von Laffert M, Helmbold P, Wohlrab J, Fiedler E, Stadie V, Marsch WC. Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. *Exp Dermatol*. 2010;19(6):533-537.
11. Zouboulis CC, Nogueira de Costa A, Fimmel S, Zouboulis KC. Apocrine glands are bystanders in hidradenitis suppurativa and their involvement in gender specific. *J Eur Acad Dermatol Venereol*. 2020. https://doi.org/10.1111/jdv.16264.
12. Musilova J, Moran B, Sweeney CM, et al. Enrichment of plasma cells in the peripheral blood and skin of patients with hidradenitis suppurativa. *J Invest Dermatol*. 2020;140(5):1091-1094.e2.
13. Hoffman LK, Tomalin LE, Schultz G, et al. Integrating the skin and blood transcriptomes and serum proteome in hidradenitis suppurativa reveals complement dysregulation and a plasma cell signature. *PLoS One*. 2018;13(9):e0203672.
14. Lacarubba F, Dall'Oglio F, Musumeci ML, Nasca MR, Micali G. Secondary comedones in a case of acne conglobata correlate with double-ended pseudocomedones in hidradenitis suppurativa. *Acta Dermatol Venereol*. 2017;97(8):969-970.
15. Boer J, Jemed GB. Mechanical stress and the development of pseudo-comedones and tunnels in hidradenitis suppurativa/acne inversa. *Exp Dermatol*. 2016;25(5):396-397.
16. Lacarrubba F, Musumeci ML, Nasca MRW, Verzi AE, Fiorentini F, Micali G. Double-ended pseudocomedones in hidradenitis suppurativa: clinical, dermoscopic, and histopathological correlation. *Acta Derm Venereol*. 2017; 97(6):763-764.
17. Higgins R, Pink A, Hunger R, Yawalkar N, Navarini AA. Generalized comedones, acne, and hidradenitis suppurativa in a patient with an *FGFR2* missense mutation. *Front Med (Lausanne)*. 2017;4:16.
18. Fontao F, von Engelbrechten M, Seilaz C, Sorg O, Saurat JH. Microcomedones in non-lesional acne prone skin new

orientations on comedogenesis and its prevention. *J Eur Acad Dermatol Venereol*. 2020;34(2):357-364.
19. Capitanio B, Lora V, Ludovici M, et al. Modulation of sebum oxidation and interleukin-1α levels associates with clinical improvement of mild comedonal acne. *J Eur Acad Dermatol Venereol*. 2014;28(12):1792-1797.
20. Antiga E, Verdelli A, Bonciani D, Bonciolini V, Caproni M, Fabbri P. Acne: a new model of immune-mediated chronic inflammatory skin disease. *G Ital Dermatol Venereol*. 2015; 150(2):247-254.
21. Ingham E, Eady A, Goodwin CE, Cove JH, Cunliffe WJ. Pro-inflammatory levels of interleukin-1 alpha-like bioactivity are present in the majority of open comedones in acne vulgaris. *J Invest Dermatol*. 1992;98(6):895-901.
22. Jeremy AHT, Holland DB, Roberts SG, Thomson KF, Cunliffe WJ. Inflammatory events are involved in acne lesion initiation. *J Invest Dermatol*. 2003;121(1):20-27.
23. Schneider MR, Paus R. Deciphering the functions of the hair follicle infundibulum in skin physiology and disease. *Cell Tissue Res*. 2014;358(3):697-704.
24. Guy R, Green MR, Kealey T. Modeling acne in vitro. *J Invest Dermatol*. 1996;106(1):176-182.
25. Jenei A, Dajnoki Z, Medgyesi B, Gáspár K, Béke G, Kinyó Á. Apocrine gland—rich skin has a non-inflammatory IL-17—related immune milieu, that turns to inflammatory IL-17—mediated disease in hidradenitis suppurativa. *J Invest Dermatol*. 2019;139(4):964-968.
26. Melnik BC, John SM, Chen W, Plewig G. T helper 17 cell/regulatory T-cell imbalance in hidradenitis suppurativa.acne inversa: the link to hair follicle dissection, obesity, smoking and autoimmune comorbidities. *Br J Dermatol*. 2018;179(2):260-272.
27. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol*. 2011;9(4):244-253.
28. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC. Topographical and temporal diversity of the human skin microbiome. *Science*. 2009;324(5931):1190-1192.
29. Grice EA. The skin microbiome: potential for novel diagnostic and therapeutic approached to cutaneous disease. *Semin Cutan Med Surg*. 2014;33(2):98-103.
30. Polak-Witka K, Rudnicka L, Bume-Peytavi U, Vogt A. The role of the microbiome in scalp hair follicle biology and disease. *Exp Dermatol*. 2019;29(3):286-294.
31. Scharschmidt TC, Vasquez KS, Pauli ML, et al. Commensal microbes and hair follicle morphogenesis coordinately drive treg migration into neonatal skin cell host. *Microbe*. 2017; 21(4):467-477.
32. Paus R, Bulfone-Paus S, Bertolini M. Hair follicle immune privilege revisited: the key to alopecia areata management. *J Invest Dermatol*. 2018;19(1):S12-S17.
33. Ring HC, Thorsen J, Saunte DM, Lilje B, Bay L, Riis PT. The follicular skin microbiome in patients with hidradenitis suppurativa and healthy controls. *JAMA Dermatol*. 2017; 153(9):897-905.
34. Ring HC, Sigsgaard V, Thorsen J, et al. The microbiome of tunnels in hidradenitis suppurativa. *J Eur Acad Dermatol Venereol*. 2019;33(9):1775-1780.
35. Brauweiler AM, Goleva E, Leung DYM. *Staphylococcus aureus* lipoteichoic acid damaged the epidermal barrier through an IL-1 mediated pathway. *J Invest Dermatol*. 2019;139(8): 1753-1761.e4.
36. Qin M, Pirouz A, Kim MH, Krutzik SR, Garbán HJ, Kim J. Propionibacterium acnes induces IL-1β secretion via the NLRP3 inflammasome in human monocytes. *J Invest Dermatol*. 2014;134(2):381-388.
37. Altmeier S, Toska A, Sparber F, Teijeira A, Halin C, Gut-Landmann SL. IL-1 coordinates the neutrophil response to C albicans in the oral mucosa. *PLoS Pathog*. 2016;12(9): e1005882.
38. Sanmiguel JC, Olaru F, Li J, Mohr E, Jensen LE. Interleukin-1 regulated keratinocyte expression of T cell targeting chemokines through interleukin-1 receptor associated kinase (IRAK-1) dependent and independent pathways Cell. *Signal*. 2009; 21(5):685-694.
39. Frew JW. ASCA antibodies in hidradenitis suppurativa: more than a gut feeling. *J Allerg Clin Immunol*. 2020. https://doi.org/10.1016/j.jaci.2020.03.022.
40. Assan F, Gottlieb J, Tubach F, et al. Anti-*Saccharomyces cerevisiae* IgG and IgA antibodies are associated with systemic inflammation and advanced disease in hidradenitis suppurativa. *J Allerg Clin Immunol*. 2020. https://doi.org/10.1016/j.jaci.2020.01.045.
41. Frew JW, Hawkes JE, Krueger JG. A systematic review and critical evaluation of immunohistochemical associations in hidradenitis suppurativa. *F100Res*. 2018;7:1923.
42. Frew JW, Hawkes JE, Krueger JG. A systematic review and critical evaluation of inflammatory cytokine associations in hidradenitis suppurativa. *F100Res*. 2018;7:1930.
43. Vossen ARJV, Ardon CB, van der Zee HH, Lubberts E, Prens EP. The anti-inflammatory potency of biologics targeting tumour necrosis factor-alpha, interleukin (IL)-17A, IL-12/23 and CD20 in hidradenitis suppurativa: an ex vivo study. *Br J Dermatol*. 2019;181(2):314-323.
44. Zouboulis CC, Nogueira da Costa A, Makrantonaki E, Hou XX, Almansouri D, Dudley JT. Alterations in innate immunity and epithelial cell differentiation are the molecular pillars of hidradenitis suppurativa. *J Eur Acad Dermatol Venereol*. 2020;34(4):846-861.
45. Shanmugan VK, Jones D, McNish S, Bendall ML, Crandall KA. Transcriptome patterns in hidradenitis suppurativa: support for the role of antimicrobial peptides and interferon pathways in disease pathogenesis. *Clin Exp Dermatol*. 2019;44(8): 882-892.
46. Wolk K, Warszawska K, Hoeflich C, et al. Deficiency of IL-22 contributes to a chronic inflammatory disease: pathogenetic mechanisms in acne inversa. *J Immunol*. 2011;186(2): 1228-1239.
47. Ghias MH, Hyde MJ, Tomalin LE, et al. Role of the complement pathway in inflammatory skin diseases: a focus on hidradenitis suppurativa. *J Invest Dermatol*. 2020;140(3):531-536.e1.
48. Grand D, Navrazhina K, Frew JW. Integrating complement into the molecular pathogenesis of hidradenitis suppurativa. *Exp Dermatol*. 2020;29(1):86-92.
49. Kanni T, Zenker O, Habel M, Riedemann N, Giamarellos-Bourboulis EJ. Complement activation in hidradenitis suppurativa: a new pathway of pathogenesis? *Br J Dermatol*. 2018; 179(2):413-419.
50. Giamarellos-Bouboulis EJ, Argyropoulou M, Kanni T, et al. Clinical efficacy of complement C5a inhibition by IFX-1 in hidradenitis suppurativa: an open-label single-arm trial in patients not eligible for adalimumab. *Br J Dermatol*. 2020. https://doi.org/10.1111/bjd.18877.
51. Shah A, Alhusayen R, Amini-Nik S. The critical role of macrophages in the pathogenesis of hidradenitis suppurativa. *Inflamm Res*. 2017;66(11):931-945.
52. Frings VG, Sennefelder H, Presser D, Goebeler M, Schmidt M. Altered NOX expression does not seem to account for epidermal NLRP3 inflammasome activation in hidradenitis suppurativa. *Br J Dermatol*. 2019;181(2):391-392.

53. Lima AL, Karl I, Giner T, et al. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. *Br J Dermatol*. 2016;174(3):514-521.
54. Marzano AV, Borghi A, Meroni PL, Cugno M. Pyoderma gangrenosum and its syndromic forms: evidence for a link with autoinflammation. *Br J Dermatol*. 2016;175(5):882-891.
55. Kurzen H, Jung EG, Hartschuh W, Moll I, Franke WW, Moll R. Forms of epithelial differentiation of draining sinus in acne inversa (hidradenitis suppurativa). *Br J Dermatol*. 1999;141(2):231-239.
56. Frew JW, Navrazhina K, Byrd AS, et al. Defining lesional, perilesional and unaffected skin in hidradenitis suppurativa: proposed recommendations for clinical trials and translational research studies. *Br J Dermatol*. 2019;181(6):1339-1341.
57. Vossen ARJV, van Straalen KR, Florencia EF, Prens EP. Lesional inflammatory profile in hidradenitis suppurativa is not solely driven by IL-1. *J Invest Dermatol*. 2020;140(7):1463-1466.e2.
58. Porter ML, Kimball AB. Comorbidities of hidradenitis suppurativa. *Semin Cutan Med Surg*. 2017;36(2):55-57.
59. Jimenez-Gallo D, de la Varga-Martinez R, Ossorio-Garcia L, Albarran-Planelles C, Rodriguez C, Linares-Barrios M. The clinical significance of increased serum proinflammatory cytokines, C-reactive protein, and erythrocyte sedimentation rate in patients with hidradenitis suppurativa. *Mediators Inflamm*. 2017;2017:2450401.
60. Kanni T, Tzanetakou V, Savva A, et al. Compartmentalized cytokine responses in hidradenitis suppurativa. *PLoS One*. 2015;10(6):e0130522.
61. Jensen P, Skov L. Psoriasis and obesity. *Dermatology*. 2016;232(6):633-639.
62. Romano S, Salustri E, Ruscitti P, Carubbi F, Penco M, Giacomelli R. Cardiovascular and metabolic comorbidities in rheumatoid arthritis. *Curr Rheumatol Rep*. 2018;20(12):81.
63. Raggi OP, Genest J, Giles JT, et al. Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. *Atherosclerosis*. 2018;276:98-108.
64. Jemec GB, Hansen U. Histology of hidradenitis suppurativa. *J Am Acad Dermatol*. 1996;34(6):994-999.
65. Alikhan A, Sayed C, Alavi A, et al. North American clinical management guidelines for hidradenitis suppurativa: a publication from the United States and Canadian Hidradenitis Suppurativa Foundations: part II topical, intralesional and systemic medical management. *J Am Acad Dermatol*. 2019;81(1):91-101.
66. Zouboulis CC, Desai N, Emtestam L, et al. European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol*. 2015;29(4):619-644.
67. Simonart T. Hidradenitis suppurativa and smoking. *J Am Acad Dermatol*. 2010;62(1):149-150.
68. Gallagher C, Kirthi S, Burke T, O'Shea D, Tobin AM. Remission of hidradenitis suppurativa after bariatric surgery. *JAAD Case Rep*. 2017;3(5):436-437.
69. Reily SM, Saltiel AR. Adapting to obesity with adipose tissue inflammation. *Nat Rev Endocrinol*. 2017;13:633-643.
70. Deng T, Lyon CJ, Bergin S, Caliguri MA, Hsueh WA. Obesity, inflammation and cancer. *Annu Rev Pathol*. 2016;11:421-449.
71. Gonçalves RB, Coletta RD, Silvério KG, et al. Impact of smoking on inflammation: overview of molecular mechanisms. *Inflamm Res*. 2011;60(5):409-424.
72. Patterson AT, Tian FT, Elston DM, Kaffenberger BH. Occluded cigarette smoke exposure causing localized chloracne-like comedones. *Dermatology*. 2015;231:322-325.
73. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. *Front Endocrinol*. 2013;4:71.
74. Kumtomrut C, Yamauchi T, Koike S, Aiba S, Yamasaki K. Androgens modulate keratinocyte differentiation indirectly through enhancing growth factor production from dermal fibroblasts. *J Dermatol Sci*. 2019;93(3):150-158.
75. Guy R, Kealey T. Modelling the infundibulum in acne. *Dermatology*. 1998;196(1):32-37.
76. Sivanand A, Gulliver WP, Josan CK, Alhusayen R, Fleming PJ. Weight loss and dietary interventions for hidradenitis suppurativa: a systematic review. *J Cutan Med Surg*. 2020;24(1):64-72.
77. Hoffman LK, Ghias MH, Lowes MA. Pathophysiology of hidradenitis suppurativa. *Semin Cutan Med Surg*. 2017;36(2):47-54.
78. Ogura H, Murakami M, Okuyama Y, et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity*. 2008;29:628-636.
79. Veldhoen M. Interleukin 17 is a chief orchestrator of immunity. *Nat Immunol*. 2017;18:612-621.
80. Ramirez-Carrozzi V, Sambandam A, Luis E, et al. IL-17C regulates the innate immune functions of epithelial cells in an autocrine manner. *Nat Immunol*. 2011;12:1159-1166.
81. Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. *Br J Dermatol*. 2020;182(4):1045-1047.
82. Witte-Handel E, Wolk K, Tsausi A, et al. The IL-1 pathway is hyperactive in hidradenitis suppurativa and contributed to skin infiltration and destruction. *J Invest Dermatol*. 2019;139(6):1294-1305.
83. Frew JW, Grand D, Navrazhina K, Krueger JG. Beyond antibodies: B cells in hidradenitis suppurativa: bystanders, contributors or therapeutic targets? *Exp Dermatol*. 2020;29(5):509-515.
84. Gauntner TD. Hormonal, stem cell and Notch signaling as possible mechanisms of disease in hidradenitis suppurativa: a systems-level transcriptomic analysis. *Br J Dermatol*. 2018;180(1):203-204.
85. Duchatelet S, Miskintye S, Delage M, et al. Low prevalence of gamma-secretase complex gene mutations in a large cohort of predominantly Caucasian patients with hidradenitis suppurativa. *J Invest Dermatol*. 2020. https://doi.org/10.1016/j.jid.2019.10.025.
86. Frew JW, Navrazhina K. In silico analysis of gamma-secretase-complex mutations in hidradenitis suppurativa demonstrates disease-specific substrate recognition and cleavage alterations. *Front Med*. 2019;6:206.
87. Xiao X, He Y, Li C, Zhang X, Xu H, Wang B. Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signaling pathways. *Br J Dermatol*. 2016;174(3):522-532.
88. Melnik BC, Plewig G. Impaired Notch signaling: the unifying mechanism explaining the pathogenesis of hidradenitis suppurativa (acne inversa). *Br J Dermatol*. 2013;168(4):876-878.
89. Melnik BC, Plewig G. Impaired Notch-MKP-1 signaling in hidradenitis suppurativa: an approach to pathogenesis by evidence from translational biology. *Exp Dermatol*. 2013;22(3):172-177.
90. Frew JW, Navrazhina K. No evidence that impaired Notch signaling differentiates hidradenitis suppurativa from other inflammatory skin diseases. *Br J Dermatol*. 2020;182(4):1042-1043.
91. He Y, Li C, Xu H, et al. AKT-dependent hyperproliferation of keratinocytes in familial hidradenitis suppurativa with a

NCSTN mutation: a potential role of defective miR-100-5p. *Br J Dermatol*. 2020;182(2):500-502.

92. Cao L, Morales-Heil DJ, Roberson EDO. Nicastrin haploinsufficiency alters expression of type 1 interferon stimulated genes: the relationship to familial hidradenitis suppurativa. *Clin Exp Dermatol*. 2019;44(4):e118-e125.
93. Yang J, Wang L, Zhang X, et al. Keratin 5-Cre driven deletion of NCSTN in acne inversa-like mouse model leads to markedly increased IL36q and SPRR2 expression. *Front Med*. 2020;14(3):305-317.
94. Gonzalez-Villanueva I, Guiterrez M, Hispan P, Betloch I, Pascual JC. Novel POFUT1 mutation associated with hidradenitis suppurativa—Dowling-Degos disease firm up a role for Notch signaling in the pathogenesis of this disorder. *Br J Dermatol*. 2018;178(4):984-986.
95. Jfri AH, O'Brien E, Litvinov I. Hidradenitis suppurativa: comprehensive review of predisposing genetic mutations and changes. *J Cutan Med Surg*. 2019;23(5):519-527.
96. Liu Y, Yen H, Chen CY, et al. Sialylation and fucosylation of epidermal growth factor receptor suppresses its dimerization and activation in lung cancer cells. *Proc Natl Acad Sci U S A*. 2011;108(28):11322-11373.
97. Frew JW, Vekic DA, Woods JA, Cains GD. Drug-associated hidradenitis suppurativa: a systematic review of case reports. *J Am Acad Dermatol*. 2018;78(1):217-219.
98. Rongioletti F. Histopathology. In: Micali G, ed. *Hidradenitis Suppurativa: A Diagnostic Atlas*. New York: Wiley; 2017.
99. Danby FW, Jemec GB, Marsch WCh, von Laffert M. Preliminary findings suggest hidradenitis suppurativa may be due to defective follicular support. *Br J Dermatol*. 2013;168(5):1034-1039.
100. Hoang VT, Trinh CT, Nguyen CH, Chansomphou V, Chansomphou V, Tran TTT. Overview of epidermoid cyst. *Eur J Radiol Open*. 2019;6:291-301.
101. Mozeika E, Pilmane M, Nürnberg BM, et al. Tumour necrosis factor-alpha and matrix metalloproteinase-2 are expressed strongly in hidradenitis suppurativa. *Acta Derm Venereol*. 2013;93(3):301-304.
102. Chuang YH, Dean D, Allen J, Dawber R, Wojnarowska F. Comparison between the expression of basement membrane zone antigens of human interfollicular epidermis and anagen hair follicle using indirect immunofluorescence. *Br J Dermatol*. 2003;149(2):274-281.
103. Frew JW, Navrazhina K, Marohn M, Lu PC, Krueger JG. Contribution of fibroblasts to tunnel formation and inflammation in hidradenitis suppurativa/acne inversa. *Exp Dermatol*. 2019;28(8):886-891.
104. Coates M, Mariottoni P, Cocoran DL, et al. The skin transcriptome in hidradenitis suppurativa uncovers an antimicrobial and sweat gland gene signature which has distinct overlap with wounded skin. *PLoS One*. 2019;14(5):e0216249.
105. Ge Y, Fuchs E. Stretching the limits: from homeostasis to stem cell plasticity in wound healing and cancer. *Nat Rev Genet*. 2018;19:311-325.
106. Navrazhina K, Frew JW, Sullivan-Whelan M, Gilleadeau P, Garcet S, Krueger JG. Epithelialized dermal tunnels drive "feed-forward" inflammation and transepithelial neutrophil migration in hidradenitis suppurativa. *J Allerg Clin Immunol*. 2020.
107. Scharl M, Rogler G. Pathophysiology of fistula formation in Crohn's disease World. *J Gastrointest Pathophysiol*. 2014;5(3):205-212.
108. Oh JW, Kloepper J, Langan EA, et al. A guide to studying human hair follicle cycling in vivo. *J Invest Dermatol*. 2016;136(1):34-44.
109. Kidacki M, Cong Z, Flamm A, Helm K, Danby FW, Nelson AM. Invasive proliferative gelatinous mass of hidradenitis suppurativa contains distinct inflammatory components. *Br J Dermatol*. 2019;181(1):192-193.
110. Ring HC, Bay L, Nilsson M, et al. Bacterial biofilm in chronic lesions of hidradenitis suppurativa. *Br J Dermatol*. 2017;176(4):993-1000.

4.2.5: Publication 2-S1

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Epithelialized tunnels are a source of inflammation in hidradenitis suppurativa

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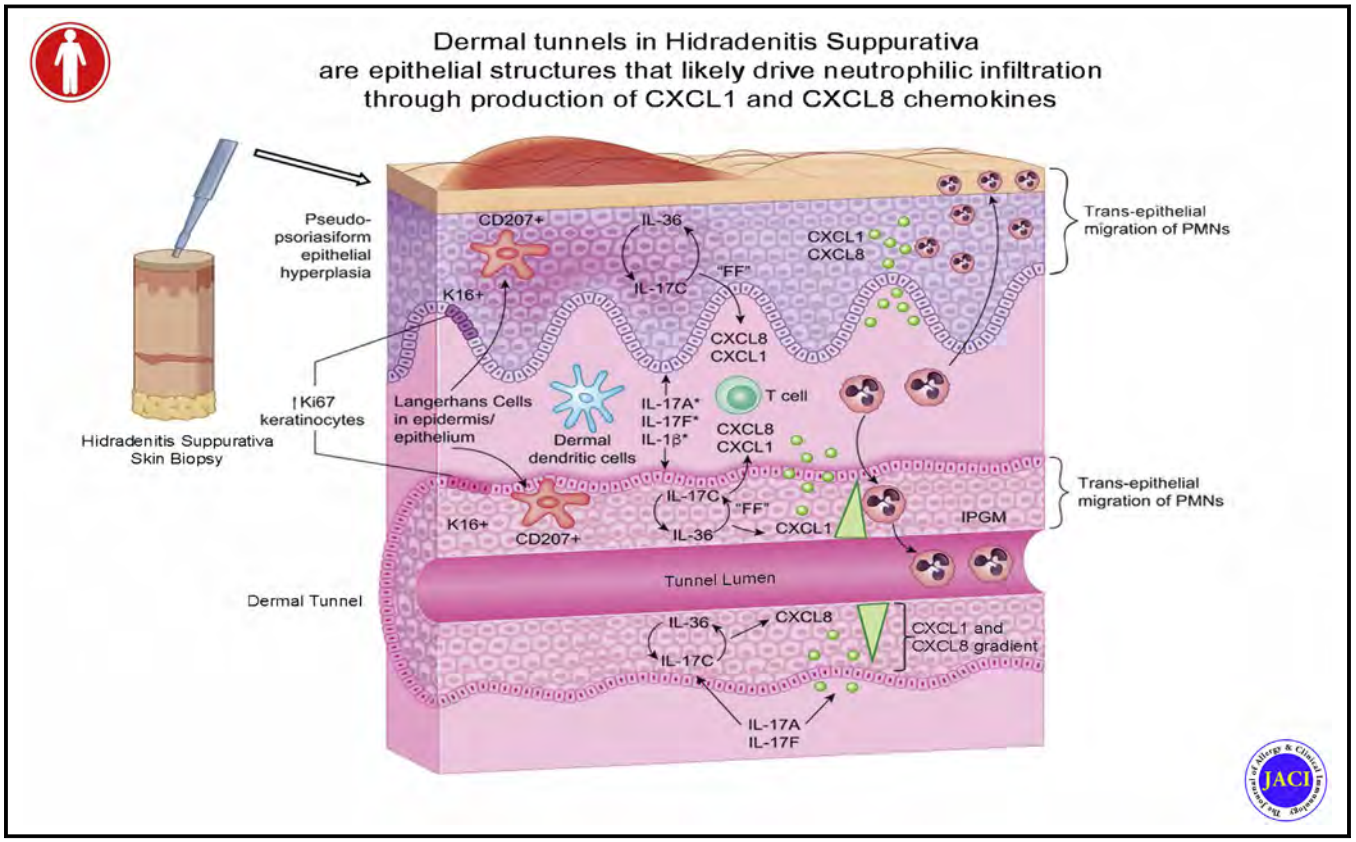
GRAPHICAL ABSTRACT



Background: Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic, painful, and burdensome inflammatory disease manifesting in nodules and abscesses, with progression to chronically draining tunnels in later-stage disease.

Objective: We sought to determine whether HS tunnels are immunologically active participants in disease activity.

Methods: Skin biopsy specimens were obtained by using ultrasound guidance in untreated patients with HS and those enrolled in an open-label study of brodalumab (ClinicalTrials.gov identifier NCT03960268) for patients with moderate-to-severe HS.

Results: Immunohistochemistry of HS biopsy specimens demonstrated that the epithelialized HS tunnels recapitulate the psoriasiform epidermal hyperplasia morphology of the

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overlying epidermis, displaying molecular inflammation, including S100A7 (psoriasin) positivity, as well as features of epidermal skin, including loricrin, filaggrin, lipocalin-2, and Melan-A positive cells. Tunnels were associated with increased infiltration of T cells, dendritic cells, and neutrophils; formation of neutrophil extracellular traps, and increased expression of psoriasiform proinflammatory cytokines. Unsupervised hierarchical clustering demonstrated a separation of HS samples based on the presence or absence of tunnels. Tunnels isolated by microdissection had higher levels of epithelium-derived inflammatory cytokines compared with the overlying epidermis and healthy controls. Clinically, the size and draining of the tunnels were decreased with treatment with the IL-17RA antagonist brodalumab.

Conclusion: These data suggest that tunnels are a source of inflammation in HS. (J Allergy Clin Immunol 2021;147:2213-24.)

Key words: *Hidradenitis suppurativa, IL-17, neutrophils, brodalumab*

Hidradenitis suppurativa (HS), also known as acne inversa, is a highly burdensome inflammatory disease manifesting in painful recurrent nodules and abscesses as well as chronically draining tunnels in more advanced (Hurley stage 2 and 3) disease.[1,2] HS is associated with high morbidity, with patients experiencing anxiety, depression, sexual dysfunction, and overall lower quality of life than with other dermatologic conditions.[3,4] Current treatments of HS include surgical resection (complicated by high rates of recurrence); antibiotics; and adalimumab, the only US Food and Drug Administration–approved biologic for HS that targets tumor necrosis factor (TNF)-α.

Despite the fact that HS is a global disease with prevalence ranging between 0.028% and 4%, understanding of its pathogenic mechanisms remains incomplete.[5-10] For one, animal models of HS demonstrate multiple barriers to high-fidelity reconstruction of human disease.[11-13] In addition, HS research is complicated by the heterogeneous morphology of the disease. Compared with other inflammatory dermatoses such as atopic dermatitis and psoriasis, HS has multiple morphologic manifestations associated with active inflammation, ranging from superficial nodules and deep abscesses to persistent draining tunnels.[14,15] The wide variation in specific cytokine levels between different studies suggests that the diverse morphologic manifestations of HS may be governed by different inflammatory pathways.[16] Therefore, to investigate variation in pathologic mechanisms between different morphologic structures in HS, investigations must rely on translational human studies.

Dermal tunnels (also known as sinus tracts or fistulae) are structures unique to HS; they have not been identified in any other inflammatory systemic skin disease.[17] Tunnels cause significant pain and morbidity[1,18] via chronic malodorous discharge, and they are predictors of poor response to existing medical therapies, including adalimumab.[1,19] Furthermore, it has been suggested that diffuse involvement or interconnected tunnels (as seen in Hurley stage 3) are associated with a more aggressive disease course.[20] Tunnels have traditionally been considered an end-stage fibrotic product of dermal inflammation, with no known contributions to inflammation in HS.[10,17,21,22] This is despite existing evidence of stratified squamous tunnel epithelium similar to the overlying epidermis.[23] and the active inflammatory characteristics of the tunnel-associated infiltrative proliferative gelatinous mass (IPGM).[24,25] The IPGM is an opaque white, reddish, or violaceous jelly-like material found in the lumen of HS tunnels; it contains a mixed population of $CD45^+$ inflammatory cells, neutrophils, macrophages, and T_H cells, as well as elevations of the levels of IL-8, IL-16, IL-1α, and IL-1β.[25] These features mirror the inflammatory characteristics of the dermal compartment in HS lesions,[16,26] suggesting a role for tunnels as active mediators of inflammation. However, the precise mechanism of IPGM formation and the cellular and molecular characteristics of HS tunnels remain incompletely described, and their potential contributions to inflammation in the disease remain unclear.

In this study, we analyze specimens from HS patients with and without tunnels, and report that dermal tunnels contribute to inflammation rather than being just an inactive end-stage feature of the disease. We demonstrate that compared with samples from patients without tunnels and samples from healthy controls, samples with HS tunnels are associated with increased levels of inflammatory infiltration and proinflammatory cytokines. We establish the potential role of tunnels in HS clinical pathogenesis by blocking IL-17 signaling with brodalumab, an anti-IL-17 receptor A (IL-17RA) antibody, and demonstrating a decrease in tunnel diameter and drainage.

Abbreviations used

H&E:	Hematoxylin and eosin
HS:	Hidradenitis suppurativa
IHC:	Immunohistochemistry
IL-17RA:	IL-17 receptor A
IPGM:	Infiltrative proliferative gelatinous mass
NET:	Neutrophil extracellular trap
NL:	Nonlesional
RT:	Room temperature
TLDA:	TaqMan Low Density Array
TNF-α:	Tumor necrosis factor alpha

METHODS

Study cohort

All data and sample collections were performed according to a protocol approved by the Rockefeller University Institutional Review Board in line with the Declaration of Helsinki. Patients were enrolled from a prospective, single-center clinical trial (ClinicalTrials.gov identifier NCT03960268) and a clinical study on natural progression of disease in HS. Only patients with HS as confirmed by clinical diagnosis by an attending dermatologist were included in the study. All patients underwent a washout period of at least 5 half-lives from previous treatment before inclusion in the study. Patients under the age of 18 years were excluded. Informed consent was obtained, and all questions were answered. We performed Doppler ultrasonography on suspected tunnels, where HS tunnels were defined as hypodermal anechoic or hypoechoic bands.[11] Ultrasound-guided punch biopsy specimens of HS skin with tunnels and HS skin without tunnels were obtained from each patient by an experienced attending dermatologist (J.W.F.) who used a previously described and validated approach.[27]

Collection of tissue samples

Patients with Hurley stage 2 or stage 3 HS were offered the option to participate in the study. Clinical examination, photography, skin ultrasonography, and skin biopsy specimens were obtained from untreated patients (N = 22)[14] (see Fig E1 in this article's Online Repository at www.jacionline.org). Visual assessment of patients was performed by an attending dermatologist. Tunnels were identified using ultrasound as previously described.[11]

Multichannel color power Doppler ultrasound (General Electric Healthcare, Chicago, Ill) with a multifrequency linear probe (13 MHz-22 MHz) was utilized. The tunnels were examined at a minimum of 2 perpendicular axes to assess both long- and-short tunnel axes. Body mass index– and site-matched healthy volunteer control specimens were included in the study (n = 9). Each collected sample was bisected, with half embedded in optimal cutting temperature (OCT) compound and the other half frozen in RNAlater RNA stabilization reagent until extraction.

IHC and quantitative cell counting

Immunohistochemistry (IHC) was performed on frozen OCT-embedded tissues according to previously published protocols.[28-30] In summary, frozen skin biopsy specimens were dried and fixed in acetone for 3 minutes. Samples were blocked in 10% normal serum corresponding to the species from which the antibody was produced. Samples were incubated overnight in primary antibody at 4°C, washed, and blocked in corresponding biotinylated secondary antibody at room temperature (RT) for 30 minutes (Vector Laboratories, Burlingame, Calif). Signal was developed using chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich, Burlington, Mass). The antibodies utilized in the study are listed in Table E1 (in the Online Repository at www.jacionline.org). Shandon eosin (Thermo Fisher Scientific, Waltham, Mass) was used to perform hematoxylin and eosin (H&E) staining. Epidermal thickness, area, and numbers of positive cells per mm^2 of epidermis and dermis were counted manually using ImageJ, V1.42 image analysis software (National Institute of Health, Bethesda, Md). Although multiple patient samples were stained, a representative image is shown in each IHC figure.

Quantitative real-time PCR

RNA from frozen skin biopsy specimens was isolated using the miRNAeasy Mini kit (Qiagen, Hilden, Germany). DNA was removed using on-column DNase digestion from the Qiagen RNase-free DNase Set (Qiagen). A subset of biopsy specimens (n = 8) confirmed to include epithelialized tunnels were microdissected for transcriptomic profiling of overlying epidermis versus epithelialized tunnels. Expression was normalized to the hARP housekeeping gene. Because tunnels are of variable sizes, the signal was normalized to the total amount of RNA extracted to accurately compare mRNA signal between HS tunnels and the overlying epidermis. Primers were generated and are listed in Table E2 and Table E3 (in the Online Repository at www.jacionline.org). TaqMan Low Density Array (TLDA) cards were utilized for larger analysis (Applied Biosystems, Foster City, Calif). The probe set selection criteria were previously reported,[30] and the data were normalized to the *Rplp0* housekeeping gene.

Statistical analysis

All statistical analysis was performed in R language (www.R-project.org; R Foundation, Vienna, Austria) using packages available through the Bioconductor project (www.bioconductor.org). All analysis was modeled using the mixed effect model. IHC markers were compared between HS biopsy specimens with and without tunnels versus specimens from healthy controls, and total counts were assessed using *lsmeans*. TLDA expression values were compared between HS biopsy specimens with and without tunnels versus nonlesional (NL) tissue. The comparison was performed using *lsmeans* of log2-transformed normalized TLDA values but adding a NL tissue and its interaction with time as a fixed-factor. Hierarchical clustering was performed on log2-transformed normalized TLDA with Euclidean distance and a McQuitty agglomeration scheme. qRT-PCR (RT-PCR) expression was analyzed between HS epidermis versus dermis and healthy controls. Comparison was performed using *lsmeans* of log2-transformed *hARP* normalized expression values. Sonographic epidermal thickness, tunnel diameter and dermal Doppler intensity were assessed by estimating a mixed-effect model with random intercept for each patient. Comparisons between groups were made for weeks 4, 12, and 24 as compared with baseline. All hypotheses and testing were conducted with contrasts under the general framework for linear models. The *P* values from the *t* tests were adjusted for multiple hypotheses by using the Benjamini-Hochberg procedure.

RESULTS

Dermal HS tunnels are clinically occult structures, but they can be clearly identified sonographically and histologically

A total of 22 patients were included in this study, as were 9 site-matched healthy volunteer controls (see Fig E1 in the Online Repository at www.jacionline.org). HS lesional skin was examined. Visual assessment of the clinical appearance of axillary (Fig 1, *A, B* and *D*), and submammary (Fig 1, *C*) regions with more severe (Hurley stage 3) disease (Fig 1, *B* and *D*) demonstrated hypertrophic scarring and dermal retraction of the superficial skin into linear cords (most prominently observed in Fig 1, *D*). Clinically, however, dermal tunnels in HS could be detected only by their superficial ostia in areas of active disease, posing challenges in locating tunnels (*white arrows* in Fig 1, *A-D*). We therefore elected to utilize sonographic assessment to identify clinically appearing tunnels for biopsy specimens (see Fig E1, *A*). Under sonographic assessment, parallel hyperechoic linear bands were identified (Fig 1, *E-H*). These appeared similar to the hyperechoic linear band of the overlying epidermis and correlated on histology with the presence of stratified squamous epithelial structures in the deep dermis (Fig 1, *I-L*). Histopathologic analysis of affected skin samples by H&E staining demonstrated the presence of deep dermal tunnels (Fig 1, *I-L*).

The epithelium of dermal tunnels recapitulates the structure of the overlying epidermis

HS epidermis has been previously characterized histologically by epidermal hyperplasia.[16,29,31,32] Consistent with this, we observed a thickened epithelium with epidermal psoriasiform hyperplasia in HS lesional skin (Fig 2, *A*). Previous studies have shown that tunnels may be lined by squamous epithelium.[23] Histologic analysis of HS tunnels by H&E staining demonstrated that tunnels are characterized by a contiguous interconnected cylinder of keratinocytes with a central lumen (Fig 2, *A*, in which *L* denotes Lumen). The more basal portions of the tunnel demonstrate interconnected rete ridges with features similar to those of the overlying psoriasiform epidermis. Intermittent loss of nuclear hematoxylin staining and the development of a glassy appearance, along with an eosin-staining hyperkeratosis, was seen in the luminal portions of the epithelium. These features were all suggestive of a keratinocyte-based epithelial structure with progressive differentiation in line with what is seen in the epidermis.

Given that histologically the epidermis of HS samples displayed psoriasiform-like hyperplasia, and that tunnels are epithelial in nature, we compared the results of immunohistochemical staining of the tunnel epithelium with markers of known positivity in the psoriasiform epidermis and hair follicle keratinocytes (Fig 2). Within the tunnels, basal and suprabasal keratinocytes were discernible, but no equivalent of the stratum granulosum or stratum spinosum was evident. Comparison with healthy skin demonstrated a significant elevation in levels of S100A7 across the luminal third portion of the epidermis comparable to the intense granular layer staining in the overlying psoriasiform epidermis (Fig 2, *B*). Keratin 16 staining was identified

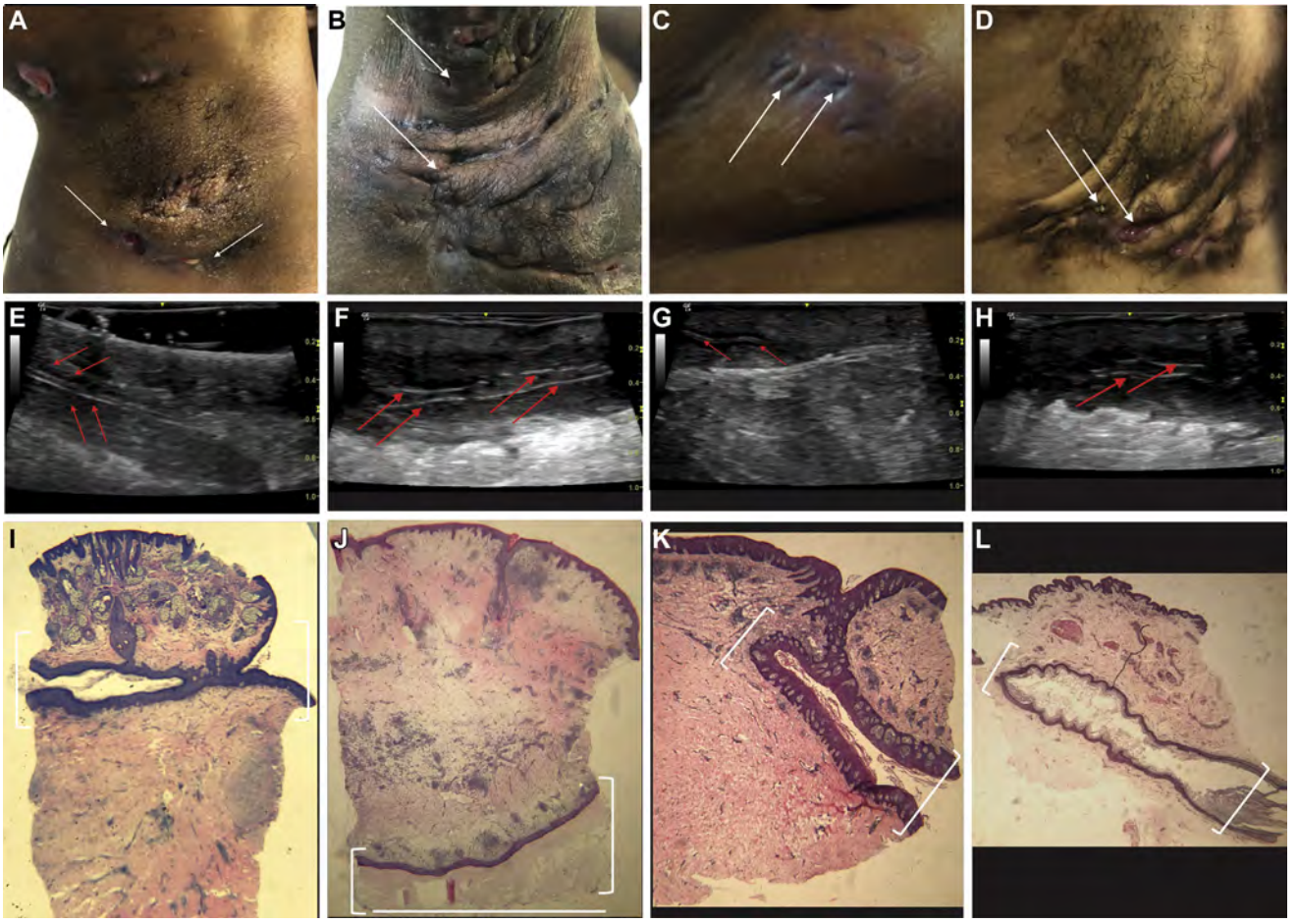

FIG 1. Ultrasonography identifies deep dermal tunnels in HS. **A-D,** Clinical assessment of tunnels marked by superficial ostia (*white arrows*): axilla **(A)**, axilla **(B)**, breast **(C)**, and axilla **(D)**. **E-H,** Corresponding ultrasound images of tunnels detected by clinical examination. Red arrows highlight the hyperechoic border of the tunnel on ultrasound imaging. **I-L,** Light microscopy of the tunnel (1.2 magnification). White brackets outline the tunnel.

throughout the entirety of the tunnel epithelium, whereas normal and psoriasiform epidermis had concentration in the basal (and less so suprabasal) keratinocytes (Fig 2, *C*). This indication of ongoing keratinocyte hyperplasia was corroborated by increased Ki67-positive staining in the basal layer of the tunnel epithelia comparable to that in the overlying psoriasiform epidermis (Fig 2, *D*). Taken together, the positive staining of S100A7 (Fig 2, *B*), keratin 16 (Fig 2, *C*), and Ki67 (Fig 2, *D*) confirmed the epithelial structures to be composed of actively proliferating keratinocytes.

Filaggrin and loricrin, both of which are essential components of the cornification of the epidermis, demonstrated different staining patterns in tunnels than in the overlying epidermis or healthy controls. Filaggrin staining (Fig 2, *E*) was inconsistent but localized to the luminal epithelium with slightly less intensity, whereas loricrin staining was in line with staining in the overlying psoriasiform epidermis. (Fig 2, *F*). This suggests an intact keratinocyte differentiation program consistent with differentiation in the overlying epidermis.

We then asked whether tunnels contained other cellular components of skin. Trichohyalin was absent from healthy controls and overlying epidermal keratinocytes but was intermittently positive in the tunnel epithelia (Fig 2, *G*). No evidence of follicular morphology was evident across the sections examined. Melanocytes were identified in the basal layer of HS tunnels by Melan-A (Fig 2, *H*) and c-Kit (Fig 2, *I*), and dermal mast cells were identified by c-Kit (Fig 2, *H*). The intraepithelial melanocyte cell populations were of density comparable to that seen in the overlying psoriasiform epidermis (Fig 2, *H*). Tunnels had increased staining of lipocalin-2, which was previously associated as a marker of IL-17–activated keratinocytes (Fig 2, *J*).[33] These results indicate that the morphologic structure of dermal tunnels recapitulates the structure of the overlying psoriasiform epidermis, with the exception of intermittent trichohyalin staining and incomplete intermittent staining of components of the cornified envelope. We termed the morphologic characteristics of the tunnels as demonstrating a pseudo-psoriasiform pattern reflecting the similarities with the overlying epidermis.

HS tunnels have increased inflammatory infiltration compared with the overlying superficial epidermis

Given that histologically, tunnels recapitulate the structure of the overlying epidermis of HS skin, we asked whether tunnels are also immunologically active. First, we inquired as to

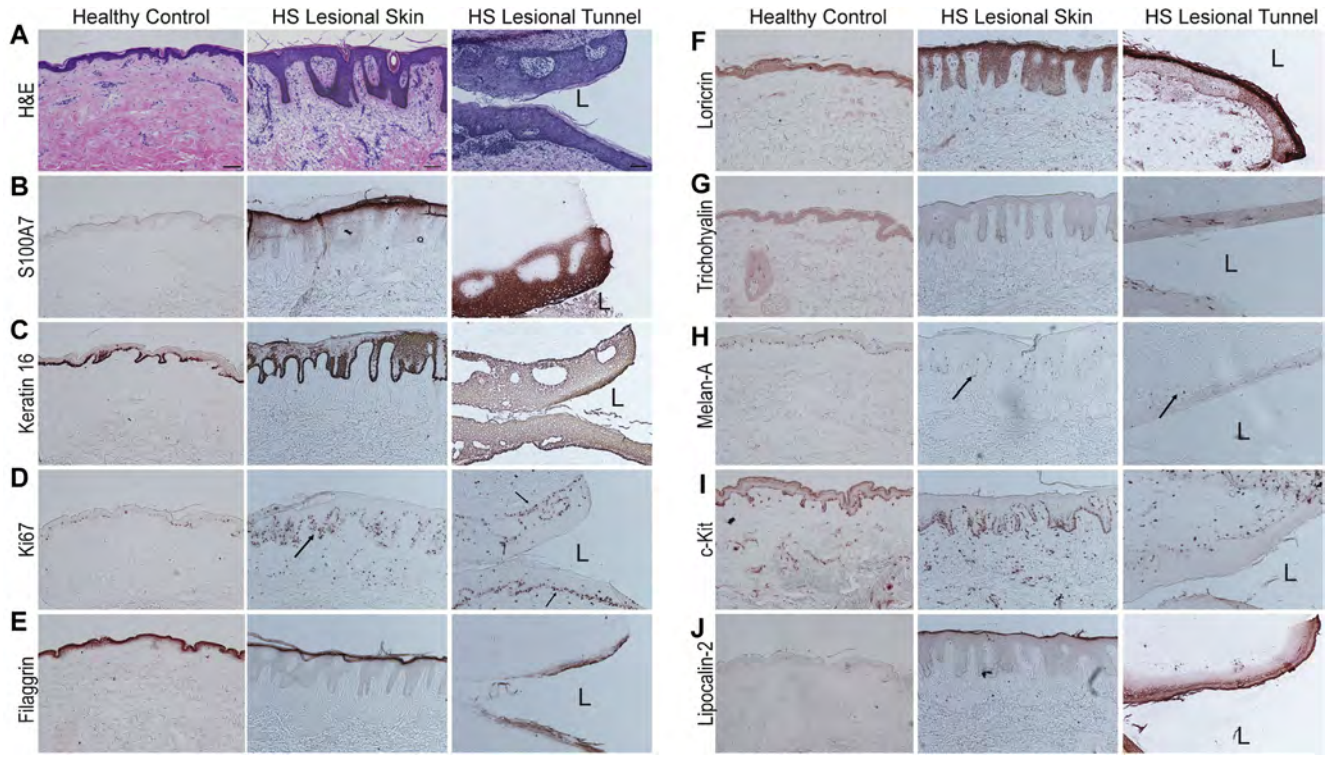

FIG 2. HS tunnels recapitulate the structural properties of the overlying epidermis. **A,** Representative biopsy specimens from patients with HS and site-matched healthy volunteers stained with H&E demonstrating prominent psoriasiform lengthening of the rete ridges, thinning of the suprapapillary plate, hyperkeratosies and parakeratosies, as well as a reduction of the granular layer in the HS epidermis compared with healthy controls. HS tunnels contain a thick stratified squamous epithelium with increasing differentiation toward the lumen (L). Scale bar = 100 μm. Tunnels are marked by (**B**) S100A7 positivity (**C**) Keratin 16 and (**D**) Ki67 staining identify this epithelium as being composed of dividing keratinocytes, with increasing differentiation toward the luminal layer compared with the more basal cells (**D** [*black arrows*]). **E** and **F,** Differentiation is indicated by filaggrin (**E**) and loricrin (**F**) staining. **G,** Intermittent positive trichohyalin staining is also observed. **H** and **I,** Other cell types within the tunnel include melanocytes (**H**), with c-Kit identifying dermal mast cells (**I**). **J,** Lipocalin-2 staining is also increased in intensity in the luminal layers of the tunnel epithelium compared with superficial HS epithelium.

whether the proinflammatory functions of epithelial keratinocytes are also intact in HS tunnels. The result of IL-36γ tract staining was highly positive in the dermal tracts (see Fig E2, *B* in the Online Repository at www.jacionline.org). We then evaluated the potential for inflammatory leukocyte signaling and migration toward epithelialized tunnels in HS. Immunohistochemical analysis demonstrated an increased T-cell ($CD3^+$), dendritic cell ($CD11c^+$), and neutrophil (neutrophil elastase $[NE]^+$) infiltration in HS samples compared with in site-matched healthy controls (Fig 3, *A*). Clusters of $CD3^+$, $CD11C^+$, and NE^+ cells were evident surrounding tunnels (Fig 3, *A*). When HS samples were subdivided based on the presence or absence of dermal tunnels (Fig 3, *B*), a significantly greater number of $CD3^+$, $CD11c^+$, and NE^+ cells were present in samples containing tunnels than in samples without tunnels ($P < .001$ for $CD3^+$ and $CD11c^+$, and $P < .05$ for NE^+ cells). We then asked which region of the skin was contributing to the differences in inflammatory infiltration. There was no difference in density of inflammatory infiltrates in the epidermis and superficial dermis; however, there was a statistically significant difference in the density of inflammatory infiltration between the deep dermis and the tunnel ($P < .001$) (Fig 3, *C*). Staining with CD177 (a neutrophil activation marker) demonstrated recruitment and transmigration of neutrophils toward the tunnel lumen (see Fig E2, *D* [*width of the black triangle depicting gradient*]). A variable CXCL1 gradient with increasing CXCL1 levels toward the tunnel lumen was observed (*width of black triangle indicating the gradient* in Fig E2, *E* and also in Fig E3, which is available in the Online Repository at www.jacionline.org). The results of staining for CXCL8 were also positive throughout the tunnel epithelium, although the gradient was less well defined (see Figs E2, *F* and E4 in the Online Repository at www.jacionline.org). It was previously shown that neutrophils are able to form web-like neutrophil extracellular traps (NETs) following exposure to microbes[34-37] and that neutrophils in HS are primed to form NETs.[36] Within the epithelialized tracts, nests of neutrophils were observed, with a dense concentration at the epithelial border of the lumen (Fig 3, *D*). Consistent with this, we observed a strong infiltration of neutrophils surrounding the tunnel lumen, with formation of NETs marked by strong NE staining (see Fig E5 in the Online Repository at www.jacionline.org). Taken together, these data suggest that the ancillary nidus of inflammatory tissue surrounding the epithelized tunnel has an inflammatory infiltration at least equal to that of the superficial dermis, which has traditionally been considered the center of inflammation in the disease.

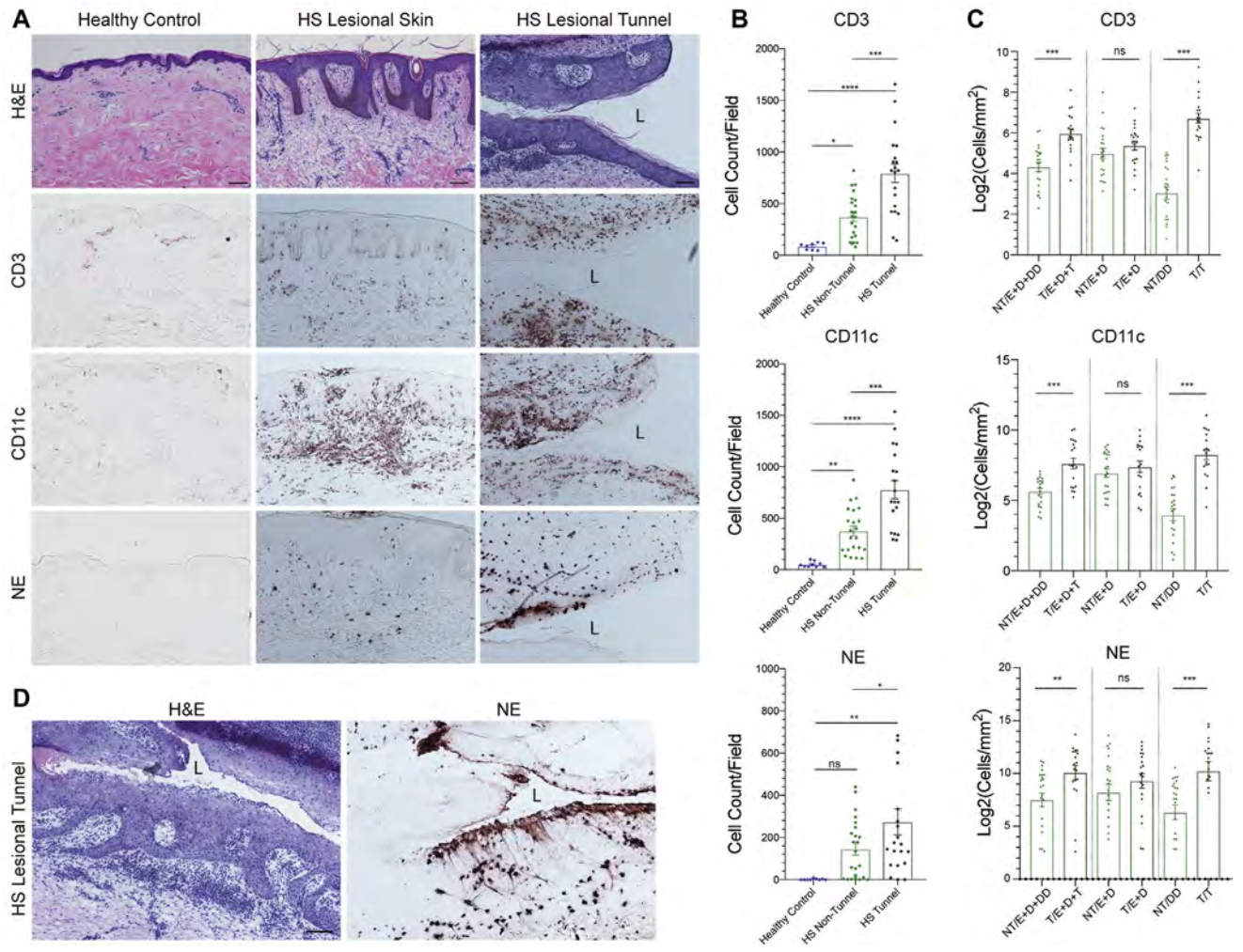

FIG 3. Tunnels are immunologically active. **A,** IHC demonstrates increased infiltration of $CD3^+$, $CD11c^+$, and NE^+ cells in HS compared with that in site-matched healthy controls. Epidermotropism and transepithelial migration toward the tunnels are also observed. Scale bar = 100 μm. **B,** Quantitative $CD3^+$, $CD11c^+$, and NE^+ cell counts highlight a significant difference in the numbers of $CD3^+$, $CD11c^+$, and NE^+ cells between HS samples with and without tunnels. **C,** Density of $CD3^+$, $CD11c^+$, and NE^+ cellular infiltrate was analyzed within nontunnel (NT) and tunnel (T) HS specimens stratified by location of cells within the biopsy specimen (with *E* indicating epidermis, *D* indicating dermis, *T* indicating tunnel, and *DD* indicating depth-matched deep dermis). There is a significant increase in inflammatory infiltration between tunnel and nontunnel specimens when the deep dermal component of biopsy specimens is taken into account. No significant elevation of $CD3^+$, $CD11c^+$, and NE^+ cell density was seen between the epidermis and the superficial dermis in tunnel and nontunnel specimens. Results are the means ± SEMs. $*P < .05$; $**P < .01$; $***P < .001$ **D,** Dense clusters of neutrophils undergoing NETosis in the tunnel epithelium adjacent to the lumen (L).

Gene expression profiling identifies HS clusters on the basis of presence or absence of tunnels

Having established the proinflammatory associations of epithelialized tunnels, we sought to explore the molecular profile of HS tissue by TLDA analysis. Unsupervised hierarchical clustering demonstrated that HS lesional skin clustered away from HS nonlesional (NL) skin, and lesional skin clustered separately based on the presence or absence of epithelialized tunnels on histologic sections (Fig 4, *A*). The results of the TLDA analysis are shown as a heatmap with fold changes relative to NL skin (Fig 4, *B*). The levels of multiple proinflammatory factors were upregulated in both tunnel and nontunnel specimens when compared with the levels in NL skin; however, the degree of elevation was much more pronounced in the tunnel samples than in the nontunnel samples (Fig 4, *B*).

The genes that demonstrated a greater upregulation in tunnel samples than in nontunnel samples included keratinocyte-derived factors (*S100A7, S100A8, S100A9*, and *LCN2*); antimicrobial factors (*DEFB4* and *IL26*); cytokines and chemokines promoting neutrophil chemotaxis (*CXCL1* and *CXCL8*), proinflammatory cytokines (*IL1β, GZMB, IL6, IL12B*, and *IL36α*), neutrophil-associated factors (*NCF1C*), and B-cell–associated cytokines and chemokines (*CD79A, TNFRSF13B*, and *IL20*). The elevation of keratinocyte-derived factors is consistent with the epithelized nature of the tunnels. Anti-inflammatory mediators. including *IL37* and macrophage migration inhibitory factor, were downregulated

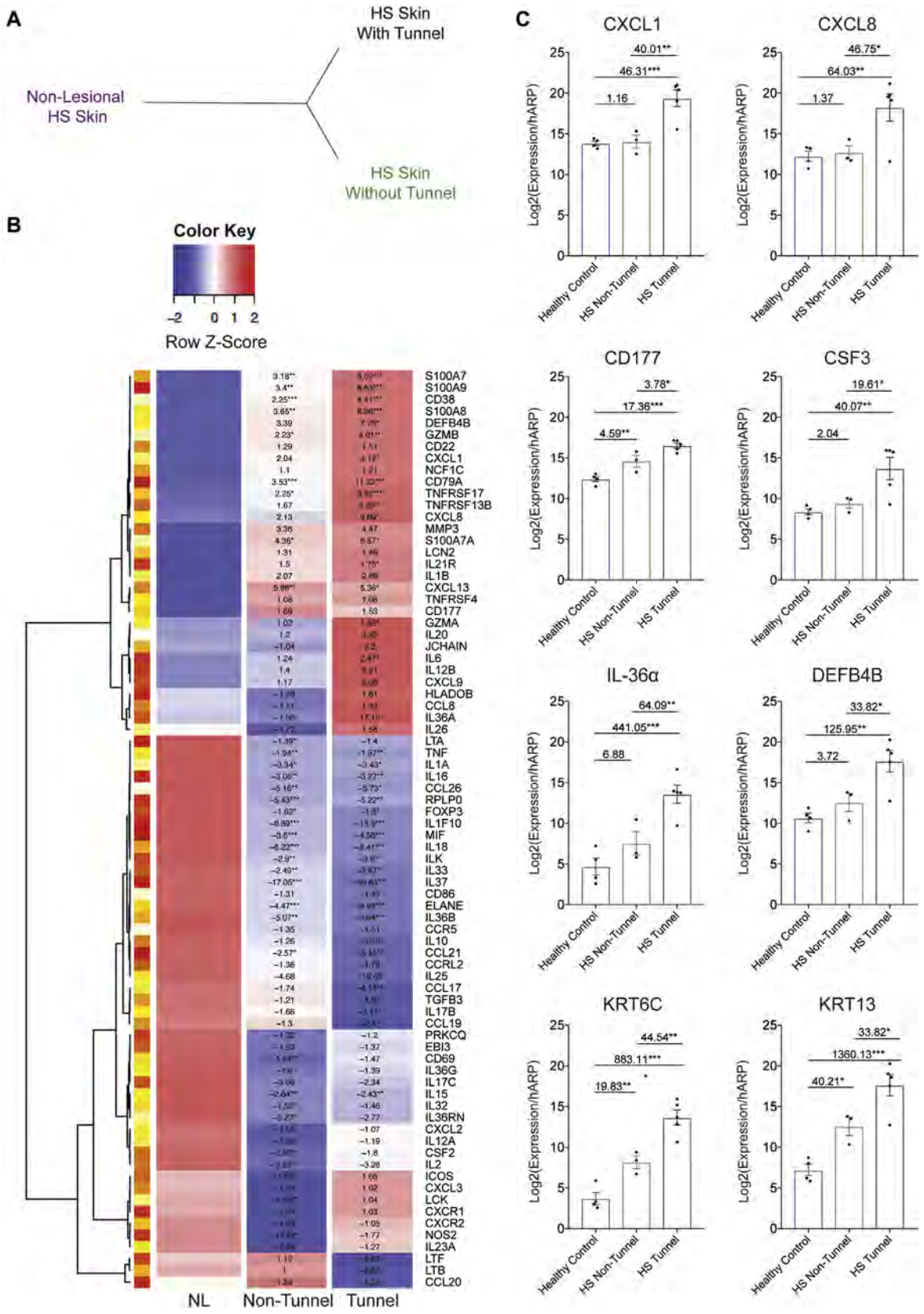

FIG 4. HS samples cluster on the basis of presence of tunnels. **A,** Unsupervised hierarchical clustering analysis of TLDA data based on the histologic presence of tunnels demonstrates distinct clustering of tunnel and nontunnel biopsy specimens compared with nonlesional (NL) tissue. **B,** Heatmap of differential gene expression of HS-associated genes in HS NL specimens (n = 7), HS samples without tunnels (n = 10), and HS samples containing tunnels (n = 6), all of which are confirmed by histologic presence of tunnels. Results indicate fold change (FCH) relative to NL. **P* < .05; ***P* < .01; ****P* < .001. **C,** Confirmatory RT-PCR of healthy controls (n = 4) and actively inflamed HS lesional samples without (n = 3) and with tunnels (n = 5). Results are the means ± SEMs. FCH is also shown. **P* < .05; ***P* < .01; ****P* < .001.

in tunnel specimens compared with in nontunnel specimens. Increased levels of expression of *CD38, CD79A, GZMA, HLADOB, IL26, JCHAIN, LCK, S100A9*, and *TNFRS17* with decreased expression levels of *CCL17* and *IL37* mRNA were observed as statistically significant between samples with and without tunnels. Taken together, these data indicate a trend toward greater upregulation of proinflammatory genes and decreased expression of anti-inflammatory genes in tunnel specimens compared with nontunnel specimens. The neutrophilic signature associated with tunnel samples was confirmed by using RT-PCR on lesional HS tissue (Fig 4, *C*). There was a statistically significant increase in the levels of epithelium-derived *CXCL1* (40.01-fold) and *CXCL8* (46.75-fold), *CD177* (activated neutrophil marker; 3.78-fold), *CSF3* (driver of increased production of neutrophils; 19.61-fold),[38] DEFB4B (a neutrophil-associated defensin peptide; 33.82-fold), and *IL-36α* (64.09-fold), as well as in the levels of *KRT6C* (44.54-fold) and *KRT13* (33.82-fold) in samples with tunnels compared to in samples without tunnels. These data suggest that samples with epithelialized tunnels have a unique inflammatory profile compared with that of samples without tunnels.

Epithelialized tunnels produce high levels of proinflammatory cytokine mRNA

On the basis of whole-tissue analysis, it is challenging to discern whether the increase in the inflammatory profile of samples with tunnels is due to the direct inflammatory contribution of tunnels or an indirect pathway of tunnels stimulating the overlying epidermis. To address the relative contributions of epithelialized tunnels and superficial epidermis and/or dermis toward inflammation in HS tissue, we microdissected HS specimens containing tunnels (confirmed histologically) to be able to isolate the superficial epidermis and superficial dermis from the deep dermis and epithelialized tunnels. RT-PCR of HS epidermis (and superficial dermis) and HS dermis (containing epithelialized tunnels) was performed to assess HS-associated inflammatory cytokines, and demonstrated higher levels of keratinocyte-derived proinflammatory mRNA in HS samples with tunnels than in samples from healthy controls (Fig 5, *A*). Given the different sizes of the tunnels (as evident in Fig 1, *I-L* and Figs E3 and E4), we normalized expression values relative to the amount of total RNA extracted (Fig 5, *B*). We detected significant elevations of the levels of *CXCL8* (27.66-fold), *IL36α* (7.4-fold), *IL17A* (8.83-fold), *IL17C* (4.57-fold), and *IL17F* (7.93-fold) in HS dermis with tunnels compared with the levels in the overlying epidermis (Fig 5, *B-D*). Elevations of the levels of *IL17C* in both the epidermis and tunnels were confirmed by IHC (Fig 5, *C*). The high levels of inflammatory and epithelium-derived cytokine mRNA detected in both the epidermis and dermal tunnels, as well as the increased levels of expression of proinflammatory cytokine mRNA in tunnels relative to in the epidermis, suggest that tunnels may contribute to inflammation in HS.

IL-17RA blockade with brodalumab decreases tunnel size and drainage in patients

Treatment with the IL-17RA antagonist brodalumab at a dose of 210 mg/1.5 mL subcutaneously every 2 weeks has been demonstrated to ameliorate the clinical manifestations of disease.[39] The impact of biologic therapy on HS tunnels has not been studied. We therefore asked whether tunnels can be modulated therapeutically. During this trial, Doppler ultrasonography of HS skin was performed at baseline, week 4, week 12, and week 24. We analyzed the tunnel size and inflammation (as measured by Doppler intensity) before and following treatment with brodalumab. In the baseline reading, a wide lumen of the tunnel (*white arrow*) and major Doppler signal were evident within the deep dermal tunnels but not in the epidermis (Fig 5, *E*). Following treatment, the tunnel wall thickness and the tunnel diameter were significantly decreased following treatment ($P < .001$) (Fig 5, *E-G*). There was also less Doppler intensity following treatment, suggesting that tunnels displayed less inflammation with IL17-RA blockade ($P < .001$) (Fig 5, *H*).

DISCUSSION

Dermal tunnels are structures unique to HS; however, the question of whether they are merely an end-stage feature of the disease or are an active inflammatory component has remained unanswered. We have characterized dermal HS tunnels and reported that they recapitulate the structure of the overlying epidermis. Tunnels are immunologically active and contribute to inflammation in HS. HS samples with tunnels have a distinct molecular profile compared with that of HS samples without tunnels. By isolating tunnels from the overlying epidermis by microdissection, we demonstrated significantly higher levels of epithelium-derived and proinflammatory cytokine mRNA in HS tunnels than in the overlying epidermis and in healthy controls. Furthermore, we have shown that the HS tunnels are at least in part dependent on IL-17 signaling, with tunnel diameter and drainage clinically decreasing in patients treated with the IL-17RA antagonist brodalumab.

HS is mediated by a complex milieu of inflammatory pathways, and the precise pathogenesis of the disease is not well understood. The T_H17 axis (including IL-17 isoforms) is considered a central feature of inflammation in the disease and has been previously characterized in HS tissues.[29,40] Cutaneous IL-17 signaling recruits neutrophils and enables their survival as well as production a myriad of IL-17–induced inflammatory mediators, including CXCL chemokines, lipocalin-2, and cathelicidin.[41,42] (Fig 2, *J* and see Fig E2, *D* and *E*). Furthermore, IL-17–derived IL-22 mediates proinflammatory effects on keratinocytes, leading to epidermal acanthosis and hyperproliferation—features that are seen in both psoriasis and HS.[43] Additionally, apocrine gland–rich skin, which is a common site for HS, has an enhanced IL-17 signature, which may partially explain the disease predilection in these anatomic regions.[44] We recently published the first report of IL-17C in HS tissue samples.[29] In this study, we have now demonstrated that epithelialized tunnels also express IL-17C. We show that the abundance of IL-17C and IL-36 in tunnel keratinocytes likely leads to increased expression of proinflammatory cytokines and chemokines, including CXCL1 and CXCL8, which are potent neutrophil chemoattractants. The increasing CD177 gradient toward the lumen of the tunnels and the formation of NETs within tunnel lumen and tunnel wall epithelium further suggest that neutrophils are activated in tunnels and are being actively recruited with marked transmigration toward the lumen of the tunnels. Furthermore, the level of mRNA of granulocyte colony-stimulating factor (CSF3), a cytokine involved in neutrophil production and release,[38] is elevated in tunnel samples compared with the levels in nontunnel samples, further giving credence to the role of neutrophilic activity in HS tunnel

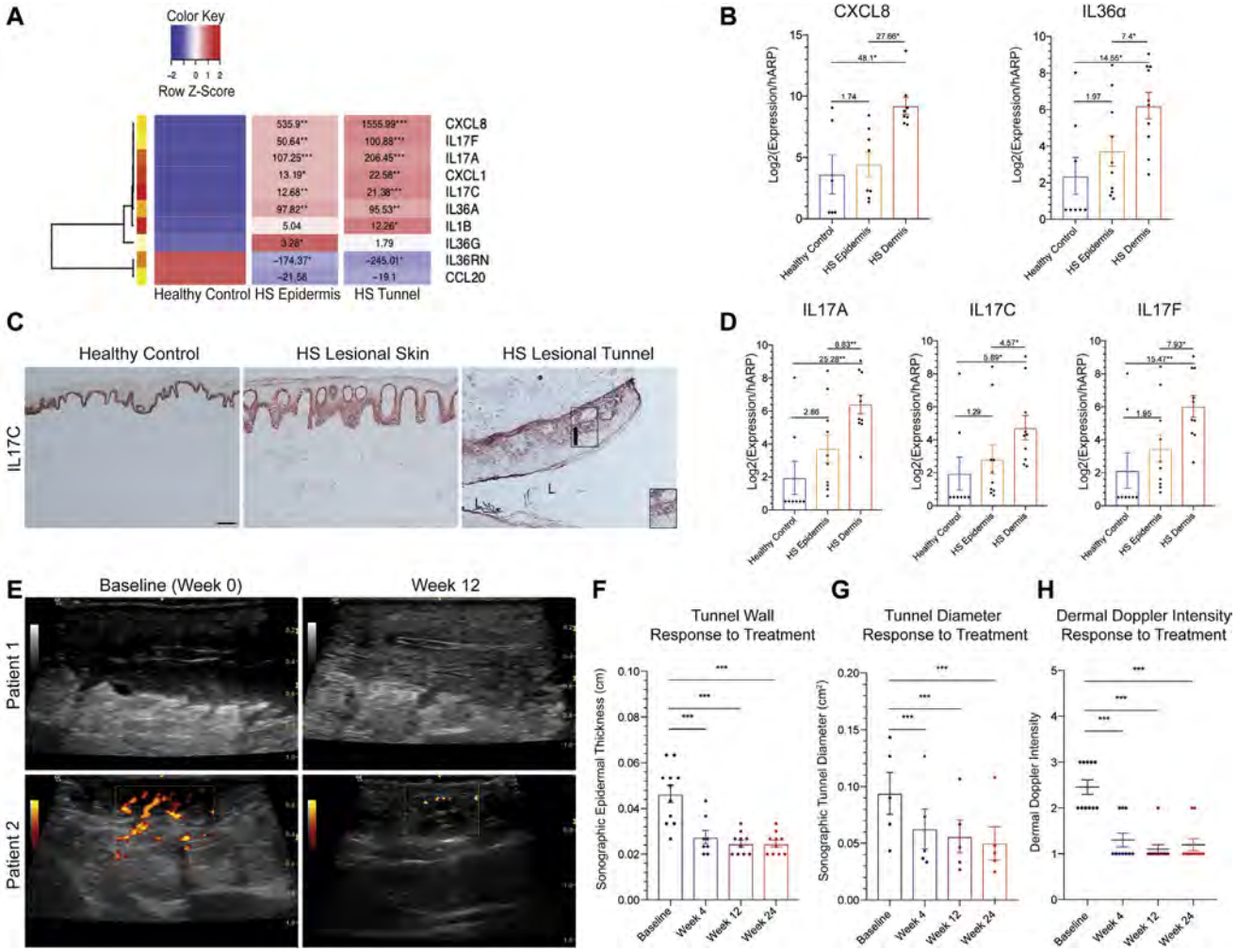

FIG 5. Tunnels are active mediators of inflammation in HS and are therapeutically targetable. **A,** Heatmap of supervised clustering of proinflammatory mediators in bisected specimens of HS skin, with the heatmap containing samples from healthy controls (n = 6), epidermis/superficial dermis (n = 8), and deep dermis containing epithelialized tunnels (n = 8). Levels of known proinflammatory mediators are highest in dermal (tunnel) specimens compared with in samples of epidermis and superficial dermis and samples from healthy controls. Fold change (FCH) is shown relative to healthy controls (with $*P < .05$; $**P < .01$; and $***P < .001$). **B,** RT-PCR demonstrates elevated levels of expression of targetable cytokines in HS dermal tunnels (HS dermis) compared with in the overlying epidermis and healthy controls, relative to the total amount of RNA recovered. There is a significant elevation of the levels of cytokines in the HS tunnels compared with in the overlying epidermis. FCH is shown. **C,** Healthy control epidermis illustrates IL-17C expression only in the basal keratinocytes. The gradient of IL-17C expression (*black arrow*) in epithelialized dermal tunnels also recapitulates the gradient seen in psoriasiform epithelium, with greatest expression in the basal layer and with reduction of expression toward the lumen (L) of the tunnels. Scale bar = 100 μm. Arrow indicates direction of the IL-17C gradient. **D,** Tunnels in HS dermis express cytokines of the IL-17 family. **E,** Doppler ultrasonography demonstrating a reduction in tunnel diameter and Doppler intensity following 12 weeks of treatment with IL-17RA antagonist brodalumab. **F-H,** There is a significant decrease in tunnel wall **(F),** tunnel diameter **(G),** and dermal Doppler intensity **(H)** following treatment with IL-17RA antagonist brodalumab. A decrease in tunnel inflammation is seen as early as 4 weeks following treatment. Results are the means ± SEMs; FCH is also shown. $*P < .05$; $**P < .01$; $***P < .001$.

pathogenesis. Given the high levels of IL-17C in pustular psoriasis, and the neutrophilic nature of HS, parallels between the 2 diseases need to be explored in the future.

Quantitative IHC allowed us to assess the association of the mixed inflammatory cellular infiltrates with epithelialized tunnels as compared with the overlying epidermis. HS samples with tunnels demonstrated greater numbers and densities of $CD3^+$, $CD11c^+$, and NE^+ cells, with the greatest change in density surrounding the epithelialized tunnels during the process of transepithelial migration. Active NETosis was also seen surrounding these tunnels to a greater degree than surrounding the overlying epidermis. The histone scaffolds associated with NETs have been identified as components of the IPGM and are thought to be due to the presence of bacterial biofilms in tunnel lumen.[25,36] Our immunohistochemical findings provide observational evidence to support epithelialized tunnels as the source of the IPGM. The CXCL1 and CXCL8 transepithelial gradient in tunnel epithelium may drive the migration of activated

neutrophils into the tunnel lumen (Figs E2, *D* and *E*, Fig 5, *C*, and see also Figs E3 and E4). This is further supported by neutrophil activation and the transmigration marker CD177 (see Fig E2, *D*). This transepithelial trafficking may occur in either the presence or the absence of a coexisting luminal biofilm. These results provide a potential mechanism for IPGM development independent of microbial biofilms.[2]

Unsupervised hierarchical clustering demonstrates that samples with tunnels clustered separately from samples without tunnels and NL tissue. The molecular signature of tunnels was significantly enhanced for keratinocyte-derived inflammatory mediators previously implicated in the pathogenesis of HS, including CXCL1, CXCL8, and DEFB4B. Additionally, the levels of B-cell–associated factors, (IL-20 and JCHAIN) were only upregulated in tunnel biopsy specimens and not in nontunnel biopsy specimens. This supports the results of Byrd et al[36] regarding the role of B cells in the disease, but it reveals that strong B-cell signals may be associated only in severe, tunnel-associated disease (which is the subset of disease that Byrd et al examined). This differential immunologic profile based on the presence or absence of tunnels in HS may explain the wide variability in tissue cytokine levels seen in the disease,[16] as stratification by disease severity and/or morphologic structures has not been routinely performed.[45]

Our investigations have characterized the structural and immunologic characteristics of epithelialized tunnels in HS lesions. Contrary to the previous pathogenic paradigm of the disease, tunnels are not merely inert, fibrotic, end-stage results of chronic inflammation.[21] We have illustrated that epithelialized tunnels recapitulate the structure of the overlying epidermis, containing not only keratinocytes but also melanocytes and Langerhans cells (Fig 2 and see Fig E2, *C*). Additionally, tunnel epithelium demonstrates pseudopsoriasiform hyperplasia and presence of a keratinocyte differentiation program similar to that seen in the overlying epidermis (Fig 2). The result of positive intermittent trichohyalin staining was 1 discrepancy seen, which is consistent with previous data on alteration in epithelial and follicular keratinocyte differentiation identified in transcriptomic data.[46] Trichohyalin staining may also be an indicator of the origin of these tunnels, given the extruding keratinocyte response seen on the outer root sheath of intact hair follicles.[31,32] However, other epithelial sources, such as eccrine and apocrine glands and ducts, would also have the potential to switch cell fate in the same way as cells of the follicular outer root sheath.[47-49] Additionally, it is unclear whether epithelial-mesenchymal transition mechanisms may be involved as part of an aberrant wound healing mechanism, as suggested in transcriptomics data on HS lesions.[50] To answer this question, further mechanistic inquiry would be necessary to faithfully ascertain the source of the cells comprising the tunnel epithelium.

The strong T_H17 cell inflammatory signature seen in specimens with epithelialized tunnels (and in the microdissected specimens containing epithelialized tunnels) suggests that tunnels may be involved in a T_H17 cell–mediated inflammation in a manner way similar to that of superficial epithelium in HS[26,29] and psoriasis vulgaris.[51] This is further supported by the strong CXCL8 signatures in bisected specimens. Although CXCL8 is produced by mononuclear phagocytic cells, as well as by fibroblasts and epithelial cells, confirmatory CXCL8 staining identified a positive staining associated with the tunnel epithelium rather than with dermal inflammatory cell infiltrates. Importantly, our data demonstrate that HS tunnels may be therapeutically targetable inflammatory structures. Treatment with the IL-17RA antagonist brodalumab, which effectively blocks the activity of all IL-17 isoforms, reduced the draining (as measured by Doppler intensity), wall thickness, and tunnel diameter in our clinical trial. HS research is hindered by the lack of animal models of HS and the limitations of *in vitro* approaches to modeling disease.[52] Although the limitation of our data is that they cannot discern whether blockade of the IL-17 signaling pathway has a direct or an indirect effect on tunnels, these data provide the first insight that tunnels are associated with IL-17 signaling.

Redefining tunnels as immunologically active structures has direct clinical relevance. Here, we have shown that patient samples with dermal tunnels demonstrate significantly greater inflammatory burden than demonstrated by samples without dermal tunnels. In a given volume of a biopsy specimen, the epithelialized tunnels produced at least the same amount of proinflammatory mediators as the superficial epidermis. Therefore, the presence of tunnels will effectively double the level of inflammation within a defined volume of skin tissue. The presence of tunnels has recently been associated with a significantly decreased odds of achieving clinical response in a reanalysis of the phase 3 clinical trials of adalimumab in HS.[53] The data that we have presented provide a molecular explanation behind this clinical observation of decreased odds of clinical response in the setting of tunnels. Standard dosing of HS therapies may successfully suppress epidermal inflammation (in the absence of tunnels), but it may be insufficient for the significantly increased level of inflammation associated with tunnels.[53] Additionally, the T_H17 cell feed-forward inflammatory loop driven by epithelium (both superficial and tunnel-associated) may reduce the likelihood of adequate inflammatory suppression with TNF-α blockade alone. It is possible that the response of the subcutaneous nodules to TNF-α blockade and not the tunnels suggests that the cellular migration to and across tunnel epithelium is more dependent on IL-17 signaling than on TNF-α signaling. Changes in inflammation and thickness of the tunnel wall demonstrate that IL-17 pathway blockade may mediate tunnel activity. We previously reported a decrease in the number of total number of nodules and abscesses in response to IL-17RA blockade, suggesting a role of IL-17 signaling in multiple HS manifestations.[39] As surface drainage of pus secretions from tunnel ostia is significantly ablated by brodalumab treatment,[39] this suggests that cellular trafficking into the lumen of dermal tunnels may be a potential mechanism promoting purulent drainage. However, whether these tunnels are able to be completely resolved by medical therapy alone is unknown. Currently, the opinion is that only surgery can remove these structures in view of their epithelialized nature. Given that surgery has high recurrence rates in patients with HS and tends to be disfiguring, thus leading to lower quality of life, novel therapies for HS are urgently needed. Furthermore, it has been suggested that HS is a progressive disease, with a diagnostic delay leading to an increased severity at presentation. Our study has uncovered a novel avenue for exploring the role of biologic therapy at an earlier stage of disease to prevent the progression to a more advanced stage and before formation of tunnels.[54] Thus, future clinical trials including patients with HS with tunnels are warranted to determine whether tunnels are potentially reversible structures.

Our study was limited by the number of patients included (N = 22), although this number was comparable to those in other studies on this disease.[36] The study included only patients with clinically advanced HS (Hurley stage 2 and 3), limiting the results to this patient group. The presented data are unable to answer the question of the origin of the tunnels in HS. Although the tunnels recapitulate the structure of the epidermis, including the ability to display psoriasiform hyperplasia, the expression of trichohyalin could suggest a follicular origin. We have provided the first evidence of the associated role of IL-17 signaling in tunnel biology, and further studies are necessary to ascertain the role of IL-17 in tunnel development and function.

Taken together, our data demonstrate that the previously uncharacterized dermal tunnels in HS are active mediators of disease pathogenesis. Unsuccessful therapeutic targeting of tunnels may explain the poor response rates to therapy by patients with HS. Blockade of IL-17RA with brodalumab leads to a clinical decrease in inflammation and size of HS tunnels, suggesting that tunnels may be associated with the IL-17 pathway. Taken together, these data demonstrate a novel avenue for development of therapeutics for this devastating disease.

We thank the members of the Krueger laboratory for technical assistance and for critical reading of the manuscript. All data, including data, documentation, and analysis available on request in writing to the corresponding author.

Key messages

- **Epithelialized tunnels in HS recapitulate the morphology, function, and proinflammatory milieu of the overlying epidermis.**
- **HS tunnels are involved in IL-17 signaling and contribute to disease pathogenesis.**
- **Clinically, HS tunnels decrease in size and draining with IL-17RA blockade.**

REFERENCES

1. Sabat R, Jemec GBE, Matusiak L, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. Nat Rev Dis Primers 2020;6:18.
2. Ring HC, Bay L, Nilsson M, Kallenbach K, Miller IM, Saunte DM, et al. Bacterial biofilm in chronic lesions of hidradenitis suppurativa. Br J Dermatol 2017;176:993-1000.
3. Deckers IE, Kimball AB. The handicap of hidradenitis suppurativa. Dermatol Clin 2016;34:17-22.
4. Gooderham M, Papp K. The psychosocial impact of hidradenitis suppurativa. J Am Acad Dermatol 2015;73(5 suppl 1):S19-22.
5. Garg A, Kirby JS, Lavian J, Lin G, Strunk A. Sex- and age-adjusted population analysis of prevalence estimates for hidradenitis suppurativa in the United States. JAMA Dermatol 2017;153:760-4.
6. Garg A, Lavian J, Lin G, Strunk A, Alloo A. Incidence of hidradenitis suppurativa in the United States: a sex- and age-adjusted population analysis. J Am Acad Dermatol 2017;77:118-22.
7. Garg A, Wertenteil S, Baltz R, Strunk A, Finelt N. Prevalence estimates for hidradenitis suppurativa among children and adolescents in the United States: a gender- and age-adjusted population analysis. J Invest Dermatol 2018;138:2152-6.
8. Zouboulis CC, Desai N, Emtestam L, Hunger RE, Ioannides D, Juhasz I, et al. European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. J Eur Acad Dermatol Venereol 2015;29:619-44.
9. Jemec GB, Kimball AB. Hidradenitis suppurativa: epidemiology and scope of the problem. J Am Acad Dermatol 2015;73(5 Suppl 1):S4-7.
10. Goldburg SR, Strober BE, Payette MJ. Part I. Hidradenitis suppurativa: epidemiology, clinical presentation, and pathogenesis. J Am Acad Dermatol 2019;82:1045-58.
11. Quartey QQ, Miller RJ, Pinsker BL, Okoh UJ, Shipman WD, George BA, et al. Lessons learned from the development of a hidradenitis suppurativa xenograft mouse model. Clin Exp Dermatol 2020;45:202-6.
12. Schneider MR, Paus R. Deciphering the functions of the hair follicle infundibulum in skin physiology and disease. Cell Tissue Res 2014;358:697-704.
13. van der Zee HH, Laman JD, Prens EP. Can animal skin diseases or current transgenic mice serve as a model for hidradenitis suppurativa? Dermatology 2012;225:9-13.
14. Saunte DML, Jemec GBE. Hidradenitis suppurativa: advances in diagnosis and treatment. JAMA 2017;318:2019-32.
15. Gill L, Williams M, Hamzavi I. Update on hidradenitis suppurativa: connecting the tracts. F1000Prime Rep 2014;6:112.
16. Frew JW, Hawkes JE, Krueger JG. A systematic review and critical evaluation of inflammatory cytokine associations in hidradenitis suppurativa. F1000Res 2018;7:1930.
17. Frew JW, Navrazhina K, Marohn M, Lu PC, Krueger JG. Contribution of fibroblasts to tunnel formation and inflammation in hidradenitis suppurativa/ acne inversa. Exp Dermatol 2019;28:886-91.
18. Ofenloch RF. Health-related quality of life in hidradenitis suppurativa. Br J Dermatol 2017;176:861-2.
19. Kimball AB, Okun MM, Williams DA, Gottlieb AB, Papp KA, Zouboulis CC, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. N Engl J Med 2016;375:422-34.
20. Vanlaerhoven A, Ardon CB, van Straalen KR, Vossen A, Prens EP, van der Zee HH. Hurley III hidradenitis suppurativa has an aggressive disease course. Dermatology 2018;234(5-6):232-3.
21. Vossen A, van der Zee HH, Prens EP. Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model. Front Immunol 2018;9:2965.
22. Goldburg SR, Strober BE, Payette MJ. Part 2. Current and emerging treatments for hidradenitis suppurativa. J Am Acad Dermatol 2019;82:1061-82.
23. Kurzen H, Jung EG, Hartschuh W, Moll I, Franke WW, Moll R. Forms of epithelial differentiation of draining sinus in acne inversa (hidradenitis suppurativa). Br J Dermatol 1999;141:231-9.
24. Margesson LJ, Danby FW. Hidradenitis suppurativa. Best Pract Res Clin Obstet Gynaecol 2014;28:1013-27.
25. Kidacki M, Cong Z, Flamm A, Helm K, Danby FW, Nelson AM. 'Invasive proliferative gelatinous mass' of hidradenitis suppurativa contains distinct inflammatory components. Br J Dermatol 2019;181:192-3.
26. Witte-Handel E, Wolk K, Tsaousi A, Irmer ML, Mossner R, Shomroni O, et al. The IL-1 pathway is hyperactive in hidradenitis suppurativa and contributes to skin infiltration and destruction. J Invest Dermatol 2019;139:1294-305.
27. Grand D, Frew JW, Navrazhina K, Krueger JG. Doppler ultrasound-based noninvasive biomarkers in hidradenitis suppurativa: evaluation of analytical and clinical validity. Br J Dermatol https://doi.org/10.1111/bjd.19343.
28. Krueger JG, Wharton KA Jr, Schlitt T, Suprun M, Torene RI, Jiang X, et al. IL-17A inhibition by secukinumab induces early clinical, histopathologic, and molecular resolution of psoriasis. J Allergy Clin Immunol 2019;144:750-63.
29. Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. Br J Dermatol 2020;182:1045-7.
30. Suarez-Farinas M, Li K, Fuentes-Duculan J, Hayden K, Brodmerkel C, Krueger JG. Expanding the psoriasis disease profile: interrogation of the skin and serum of patients with moderate-to-severe psoriasis. J Invest Dermatol 2012;132:2552-64.
31. von Laffert M, Stadie V, Wohlrab J, Marsch WC. Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae. Br J Dermatol 2011;164:367-71.
32. von Laffert M, Helmbold P, Wohlrab J, Fiedler E, Stadie V, Marsch WC. Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. Exp Dermatol 2010;19:533-7.
33. Wolk K, Wenzel J, Tsaousi A, Witte-Handel E, Babel N, Zelenak C, et al. Lipocalin-2 is expressed by activated granulocytes and keratinocytes in affected skin and reflects disease activity in acne inversa/hidradenitis suppurativa. Br J Dermatol 2017;177:1385-93.
34. Juneau RA, Pang B, Weimer KE, Armbruster CE, Swords WE. Nontypeable Haemophilus influenzae initiates formation of neutrophil extracellular traps. Infect Immun 2011;79:431-8.
35. Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Kockritz-Blickwede M. Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. J Innate Immun 2010;2:576-86.
36. Byrd AS, Carmona-Rivera C, O'Neil LJ, Carlucci PM, Cisar C, Rosenberg AZ, et al. Neutrophil extracellular traps, B cells, and type I interferons contribute to immune dysregulation in hidradenitis suppurativa. Sci Transl Med 2019;11:eaav5908.

37. Byrd AS, O'Brien XM, Johnson CM, Lavigne LM, Reichner JS. An extracellular matrix-based mechanism of rapid neutrophil extracellular trap formation in response to Candida albicans. J Immunol 2013;190: 4136-48.
38. Semerad CL, Liu F, Gregory AD, Stumpf K, Link DC. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. Immunity 2002; 17:413-23.
39. Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, et al. The effect of subcutaneous brodalumab on clinical disease activity in hidradenitis suppurativa: an open-label cohort study. J Am Acad Dermatol 2020;83: 1341-8.
40. Schlapbach C, Hanni T, Yawalkar N, Hunger RE. Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. J Am Acad Dermatol 2011;65: 790-8.
41. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485-517.
42. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004;21:467-76.
43. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J Invest Dermatol 2009;129:1339-50.
44. Jenei A, Dajnoki Z, Medgyesi B, Gaspar K, Beke G, Kinyo A, et al. Apocrine gland-rich skin has a non-inflammatory il-17-related immune milieu, that turns to inflammatory IL-17-mediated disease in hidradenitis suppurativa. J Invest Dermatol 2019;139:964-8.
45. Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR, Kirby JS, et al. Defining lesional, perilesional and unaffected skin in hidradenitis suppurativa: proposed recommendations for clinical trials and translational research studies. Br J Dermatol 2019;181:1339-41.
46. Zouboulis CC, Nogueira da Costa A, Makrantonaki E, Hou XX, Almansouri D, Dudley JT, et al. Alterations in innate immunity and epithelial cell differentiation are the molecular pillars of hidradenitis suppurativa. J Eur Acad Dermatol Venereol 2020;34:846-61.
47. Folgueras AR, Guo X, Pasolli HA, Stokes N, Polak L, Zheng D, et al. Architectural niche organization by LHX2 is linked to hair follicle stem cell function. Cell Stem Cell 2013;13:314-27.
48. Kadaja M, Keyes BE, Lin M, Pasolli HA, Genander M, Polak L, et al. SOX9: a stem cell transcriptional regulator of secreted niche signaling factors. Genes Dev 2014;28:328-41.
49. Ge Y, Gomez NC, Adam RC, Nikolova M, Yang H, Verma A, et al. Stem cell lineage infidelity drives wound repair and cancer. Cell 2017;169:636-50.e14.
50. Coates M, Mariottoni P, Corcoran DL, Kirshner HF, Jaleel T, Brown DA, et al. The skin transcriptome in hidradenitis suppurativa uncovers an antimicrobial and sweat gland gene signature which has distinct overlap with wounded skin. PLoS One 2019;14:e0216249.
51. Hawkes JE, Yan BY, Chan TC, Krueger JG. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. J Immunol 2018;201:1605-13.
52. Frew JW, Piguet V. Ex vivo models and interpretation of mechanistic studies in hidradenitis suppurativa. J Invest Dermatol 2020;140:1323-6.
53. Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, et al. Clinical response rates, placebo response rates, and significantly associated covariates are dependent on choice of outcome measure in hidradenitis suppurativa: a post hoc analysis of PIONEER 1 and 2 individual patient data. J Am Acad Dermatol 2020;82:1150-7.
54. Kokolakis G, Wolk K, Schneider-Burrus S, Kalus S, Barbus S, Gomis-Kleindienst S, et al. Delayed diagnosis of hidradenitis suppurativa and its effect on patients and healthcare system. Dermatology 2020;236:421-30.

4.2.6: Publication 2-S2

Navrazhina K, Garcet S, Gonzales J, Grand D, Frew JW, Krueger JG In-Depth Analysis of the Hidradenitis Suppurativa Serum Proteome Identified Distinct Inflammatory Subtypes. J Invest Dermatol 2021; doi:10.1016/j.jid.2021.02.742

In-Depth Analysis of the Hidradenitis Suppurativa Serum Proteome Identifies Distinct Inflammatory Subtypes

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Hidradenitis suppurativa is a chronic inflammatory dermatosis with presentations ranging from painful nodules and abscesses to draining tunnels. Using an unbiased proteomics approach, we assessed cardiovascular-, cardiometabolic-, and inflammation-related biomarkers in the serum of patients with moderate-to-severe hidradenitis suppurativa. The serum of patients with hidradenitis suppurativa clustered separately from that of healthy controls and had an upregulation of neutrophil-related markers (Cathepsin D, IL-17A, CXCL1). Patients with histologically diagnosed dermal tunnels had higher serum lipocalin-2 levels compared with those without tunnels. Consistent with this, patients with tunnels had a more neutrophilic-rich serum signature, marked by Cathepsin D, IL-17A, and IL-17D alterations. There was a significant serum–skin correlation between proteins in the serum and the corresponding mRNA expression in skin biopsies, with healthy-appearing perilesional skin demonstrating a significant correlation with neutrophil-related proteins in the serum. *CSF3* mRNA levels in lesional skin significantly correlated with neutrophil-related proteins in the serum, suggesting that *CFS3* in the skin may be a driver of neutrophilic inflammation. Clinical significantly correlated with the levels of lipocalin-2 and IL-17A in the serum. Using an unbiased, large-scale proteomic approach, we demonstrate that hidradenitis suppurativa is a systemic neutrophilic dermatosis, with a specific molecular signature associated with the presence of dermal tunnels.

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INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic inflammatory disease with an estimated prevalence of 1% (Jemec and Kimball, 2015; Jemec et al., 1996; Sabat et al., 2020). HS has a wide spectrum of clinical presentations, ranging from inflamed nodules and abscesses to interconnecting draining tunnels and late-stage fibrotic disease. Patients with HS face multiple comorbidities, including inflammatory bowel disease, depression, sexual dysfunction, and an increased risk of cardiovascular disease and metabolic syndrome (Kurek et al., 2013; Matusiak et al., 2010; Miller et al., 2014; Reddy et al., 2020; Sabat et al., 2012; van der Zee et al., 2014). Current treatment options for HS have limited efficacy (Frew et al., 2020) and are hindered by a lack of blood and serum biomarkers for assessment of inflammatory activity and therapeutic response.

Although the exact pathogenesis of HS remains unclear, recent studies have led to a paradigm shift from the traditional model of follicular occlusion as a driver of the disease to appreciating HS as a systemic inflammatory disorder with alterations involving plasma cells and B cells (Gudjonsson et al., 2020; Lowe et al., 2020); neutrophils (Byrd et al., 2019); dendritic cells (Lowe et al., 2020); macrophages (Byrd et al., 2018; Thomi et al., 2018); and multiple other proinflammatory axes, including the T helper type 17 pathway (Navrazhina et al., 2020; Wolk et al., 2011). However, most of this work has been based on histological and transcriptomic profiling of skin biopsies. Several studies have examined the serum proteome to identify potential disease biomarkers, with varying results regarding the abundance of these cytokines and proteins compared with healthy controls (Blok et al., 2016; Jiménez-Gallo et al., 2017; Vossen et al., 2019; Wolk et al., 2017). A study assessing the in-depth proteomic profile of HS serum to identify the biomarkers of disease is lacking.

Multiple studies have utilized the Olink broad proteomic panels to gain molecular insight into the disease activity in the serum of patients with inflammatory dermatoses, including atopic dermatitis (AD) (Brunner et al., 2019, 2017), alopecia areata (Glickman et al., 2021), and psoriasis vulgaris, as well as for biomarkers of therapeutic response in psoriasis (Kim et al., 2018). In addition to an increase in inflammatory proteins, these studies have identified alterations in cardiovascular biomarkers, consistent with the systemic inflammation associated with these disorders. In this study, we aimed to evaluate protein expression in HS serum.

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Abbreviations: AD, atopic dermatitis; BMI, body mass index; DEP, differentially expressed protein; HS, hidradenitis suppurativa; LCN2, lipocalin-2; LS, lesional; PL, perilesional; QC, quality control

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RESULTS

The proteomic profile of HS serum is molecularly distinct from that of healthy controls and other systemic inflammatory dermatoses

Using the Olink Proteomics platform (Uppsala, Sweden), we assessed the serum proteome of patients with Hurley II and III HS (n = 22) and body mass index (BMI)–matched healthy control individuals (n = 9) using the inflammation (92 biomarkers), cardiometabolic (92 biomarkers), cardiovascular II (92 biomarkers), and cardiovascular III (92 biomarkers) panels. Patient demographics can be found in Supplementary Table S1. Principal component analysis demonstrated that HS samples clustered separately from healthy volunteers (Figure 1a; Supplementary Figure S1). An unsupervised two-dimensional hierarchical clustering algorithm was used to group the samples on the basis of differentially expressed proteins (DEPs, defined as absolute values of [fold change] $\geq$ 1.2 and $P \leq 0.05$) (Figure 1b). HS serum had a significant increase in neutrophil-related proteins (IL-17A, CXCL1, Cathepsin D), mediators of atherosclerosis (HGF), chemotactic cytokines and receptors (CCL5, IL-4RA), and GFs (TGF-α, HGF, HB-EGF) (Figure 1b) (Bell et al., 2018). HS exhibited elevations of ST2 protein, a biomarker of cardiovascular stress and fibrosis that is a potential predictor for outcomes in patients with heart failure (Villacorta and Maisel, 2016). This finding is consistent with the increased risk of cardiovascular complications in patients with HS (Miller et al., 2014; Reddy et al., 2020).

We then conducted an enrichment analysis of DEPs for Gene Ontology biological processes terms. Pathways that were significantly enriched in the serum of patients with HS relative to that of healthy volunteers are shown in Figure 1c, with the vertical line demonstrating a false discovery rate of 0.05. The most significantly enriched pathways were related to general immune response (positive chemotaxis, chemokine-mediated signaling pathway, lymphocyte chemotaxis, inflammatory response, immune response, signal transduction) and neutrophil-mediated inflammation (neutrophil chemotaxis, neutrophil degranulation). Because the serum contains secreted proteins, we assessed the cellular structures in which the DEPs are localized to function. Protein annotation through evolutionary relationship statistical overrepresentation test for Gene Ontology cellular component terms identified tertiary granule lumen ($P = 3.95E-05$), specific granule lumen (neutrophil associated) ($P = 5.57E-05$), and extracellular space ($P = 9.42E-13$) as the cellular locations in which HS-specific proteins functioned (Mi et al., 2019) (Figure 1d). Given that smoking may be associated with HS, we performed a sensitivity analysis to account for smoking status. There were seven proteins differentially expressed between HS smokers and nonsmokers (SLAMF7, QPCT, CHIL1, SELE, NCAM1, MB, and SERPINA7). None of these proteins were differentially expressed between patients with HS and healthy controls regardless of the smoking status.

Because we observed systemic inflammation in HS serum, we compared the HS serum proteome with previously published Olink cardiovascular and inflammation panels in AD and psoriasis vulgaris (Brunner et al., 2017) using absolute value (fold change) $\geq$ 1.2 and $P \leq 0.05$ (Figure 1e). All three dermatoses had an upregulation of mediators involved in atherosclerosis (HGF) (Bell et al., 2018). Compared with AD and psoriasis, HS only had 11 unique DEPs. HS was characterized by a significant upregulation of proteins related to neutrophil chemotaxis (CXCL1) and biomarkers of cardiovascular disease (ST2), with downregulation of IL-17D. HS was more akin to psoriasis, with an upregulation of T helper type 17 pathway (IL-17A) and neutrophil-related proteins (IL-17A, Cathepsin D, CCL24). Both HS and AD had an upregulation of GF TGF-α and IL-4 immune signaling proteins (IL4-RA, SLAMF1).

LCN2 differentiates HS subtypes in serum

Lipocalin-2 (LCN2), also known as neutrophil gelatinase–associated lipocalin, has been suggested as a potential biomarker in HS, with reports identifying elevated levels of LCN2 in serum of patients with HS and palmoplantar pustular psoriasis (Wolk et al., 2018, 2017). LCN2 is a potent chemoattractant for neutrophils, promoting adhesion and extravasation of granulocytes (Schroll et al., 2012). LCN2 can also be used to measure inflammation in the context of inflammatory bowel disease (Chassaing et al., 2012; Thorsvik et al., 2017). Given the neutrophilic signature detected in HS serum and the applicability of LCN2 as a biomarker of inflammatory disease, we asked whether LCN2 is elevated in our HS cohort. Unsupervised two-dimensional hierarchical clustering of all samples arranged by increasing LCN2 levels identified two HS subgroups: a subset of patients with HS with high LCN2 levels and a subset of patients with HS with low LCN2 levels, which clustered more closely with healthy controls (Figure 2a). We identified a node of the dendrogram that was associated with increasing levels of LCN2 (black box, Figure 2a). This cluster identified proteins directly proportional to LCN2 levels in the serum, including neutrophil-related proteins (AZU1, MPO, EN-RAGE, DEFA1, CEACAM8, matrix metalloproteinase 9, CXCL8) (Figure 2b and c). Phylogenetic tree clustering of all the samples demonstrated two distinct subtypes of HS on the basis of high or low LCN2 levels in the serum (Figure 2d). We then evaluated the clinical and histological parameters associated with patients in each subgroup. The majority of the patients in the LCN2-high subgroup had histologically diagnosed dermal HS tunnels (on the basis of ultrasound examination of HS skin as well as on the basis of the presence of a visible tunnel on the histological assessment of the biopsy) compared with those in the LCN2-low subgroup, in which patients did not have histologically diagnosed tunnels. Because the criteria of histologically confirmed dermal tunnels were used, it is plausible that a patient may have had a tunnel that was missed by the punch biopsy, thus explaining the two outliers in the cohort (Figure 2d).

HS patients with tunnels have a different serum proteomic profile than patients without tunnels

We then compared the DEPs in the sera of HS patients with and without histologically confirmed tunnels, with fold change relative to healthy controls shown (Figure 3a), and also conducted an enrichment analysis of the DEPs unique to

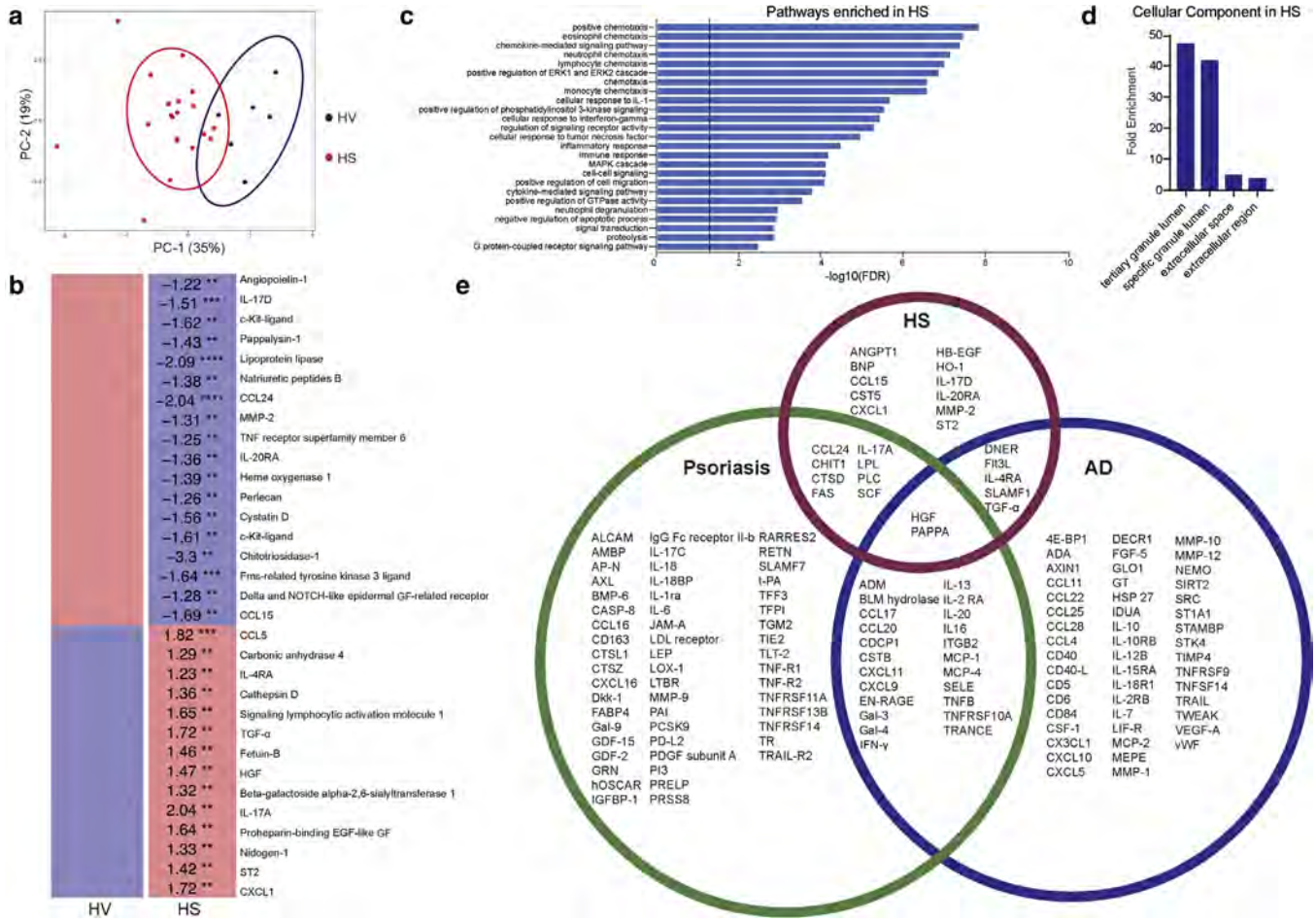

Figure 1. HS serum is molecularly distinct from that of healthy controls and other systemic skin diseases. (**a**) Principal component analysis and (**b**) unsupervised hierarchical clustering of all differentially expressed proteins (abs [FCH] $\geq$ 1.2, and $P \leq 0.05$) between HS serum and that of HV controls. FCHs relative to healthy controls are shown; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$. c-Kit ligand is found in both inflammation and cardiovascular panel. (**c**) GO biological process pathway enrichment analysis of differentially expressed proteins in HS serum relative to HV serum using the XGR tool. The vertical line shows FDR = 0.05. (**d**) Protein annotation through evolutionary relationship statistical over-representation test for GO cellular component for differentially expressed proteins in HS serum relative to HV serum (**e**) Venn diagram of differentially expressed proteins between HS, psoriasis, and AD. abs, absolute value; AD, atopic dermatitis; ERK, extracellular signal−regulated kinase; FCH, fold change; FDR, false discovery rate; GO, Gene Ontology; GTPase, guanosine triphosphatase; HS, hidradenitis suppurativa; HV, healthy volunteer; MMP, matrix metalloproteinase; PC, principal component; XGR, eXploring Genomic Relations.

HS samples with tunnels (relative to healthy controls) using the canonical, Kyoto Encyclopedia of Genes and Genomes, Reactome, and bioCarta pathways. Serum of HS patients with tunnels had an enrichment of pathways involved in proliferation and signal transduction, extracellular matrix remodeling, and tissue development (development biology, axon guidance, pathways in cancer) (Figure 3b). There were 41 unique DEPs in tunnel samples compared with 23 proteins unique to the nontunnel samples, both relative to healthy controls (Figure 3c). There was minimal overlap between tunnel and nontunnel samples (five proteins). Smoking status did not influence the serum proteome between patients with and those without tunnels; of the seven DEPs between HS smokers and nonsmokers, only one (SERPINA7) was differentially expressed between tunnel and nontunnel HS samples.

HS samples with tunnels had a neutrophilic signature (Cathepsin D, IL-17A, IL-17D, LCN2) compared with HS samples without tunnels. Serum of HS patients with tunnels had an upregulation of cardiovascular-associated biomarkers (HGF, ST2, PGLYRP1). Given the neutrophilic signature associated with tunnels and that pus draining from tunnels is neutrophil mediated, we asked whether patients with draining or nondraining tunnels had a different serum proteome profile (Figure 3d). There was a significant difference in the levels of neutrophilic-related proteins (IL-17A, LCN2, CXCL8, EN-RAGE, DEFA1, matrix metalloproteinase 9) between HS samples with draining and nondraining tunnels. In cases where there was no significant difference in the protein levels between the healthy volunteers and HS patients or between tunnel and nontunnel samples, there was a significant difference in the protein levels between samples with draining and nondraining tunnels (EN-RAGE, DEFA1, matrix metalloproteinase 9). This suggests that patients with actively draining tunnels have a different serum proteome profile. Furthermore, cardiovascular biomarker ST2 was significantly elevated in patients with draining tunnels, further linking the role of tunnels and the increased risk for cardiovascular comorbidities in HS (Figure 3d).

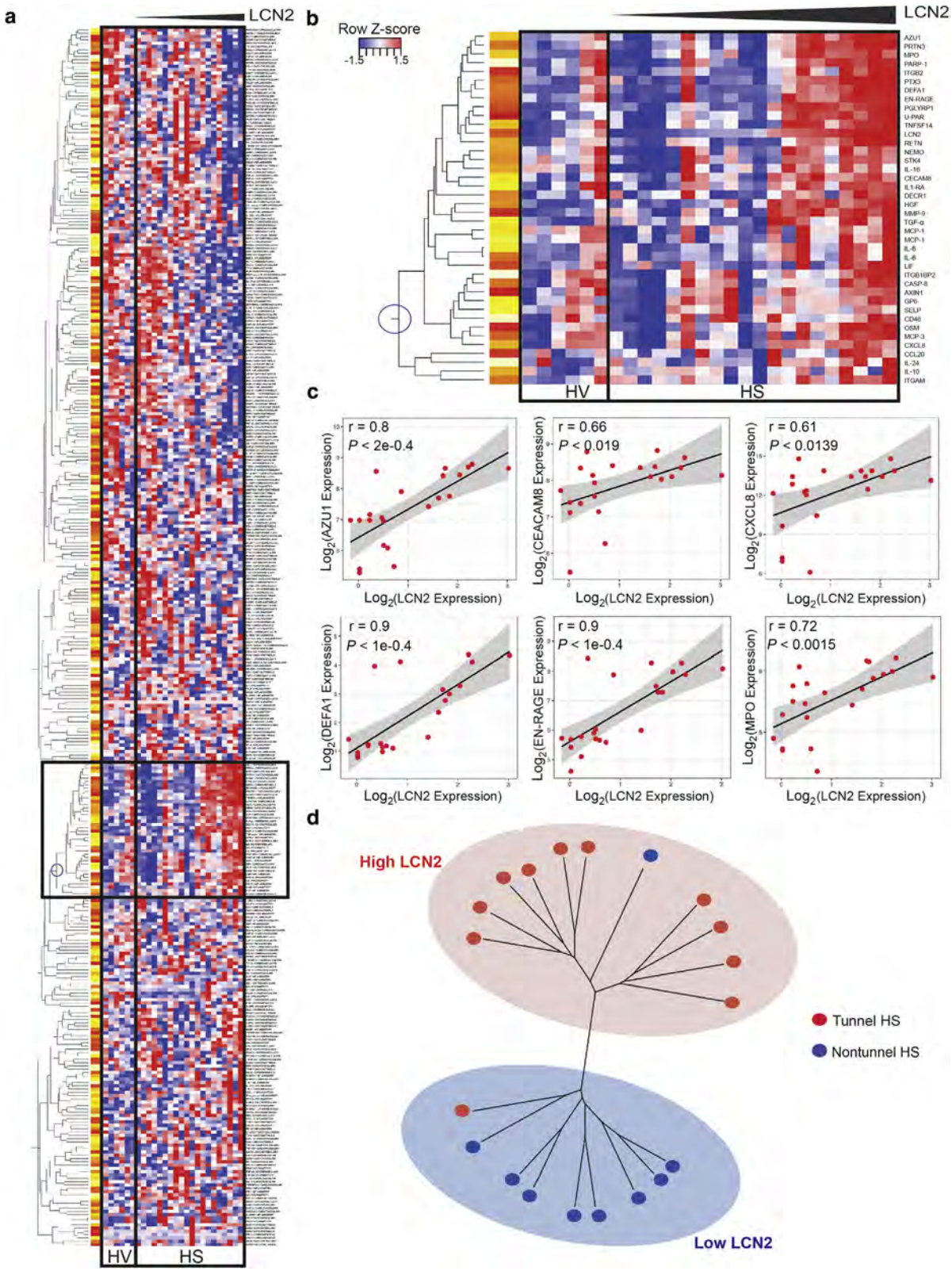

Figure 2. Serum LCN2 levels differentiate HS into two subgroups. (**a**) Unsupervised hierarchical clustering of all proteins in HS HV control serum arranged by increasing levels of LCN2. Red indicates the upregulated protein expression levels, and blue indicates the downregulated protein expression levels. The blue circle demonstrates the node of interest on the dendrogram, with the black box identifying a cluster of proteins that are directly proportional to LCN2 levels. (**b**) Magnification of the LCN2-related cluster of proteins identifies a clear demarcation of two HS subgroups on the basis of the protein level of LCN2 in serum. (**c**) Pearson correlation between expression of LCN2 and other neutrophil-related proteins in HS serum. r is Pearson correlation. (**d**) High LCN2 subgroup consists of patients with histologically diagnosed tunnels, with serum samples from this subgroup clustering separately and away from those of patients without tunnels and low LCN2 levels. HS, hidradenitis suppurativa; HV, healthy volunteer; MMP, matrix metalloproteinase.

Correlation of biomarkers suggests a skin–blood interaction in HS

We examined lesional (LS), and healthy-appearing perilesional (PL) and nonlesional skin biopsies as previously described (Frew et al., 2019) (Figure 4a). We first asked whether there was any correlation between HS skin and serum by studying IL-6, which has been previously suggested as a biomarker in HS serum (Jiménez-Gallo et al., 2017). IL-6 protein level in the serum was significantly correlated with *IL-6* mRNA in LS ($r = 0.62$, $P = 0.0096$), PL ($r = 0.56$, $P = 0.0138$), and nonlesional ($r = 0.5$, $P = 0.0261$) skin (Figure 4b). We focused further analysis on PL skin biopsies because the overall RNA quality was better in PL samples than in LS samples, consistent with the increased presence of neutrophils in lesions of LS skin. There was a significant correlation between the proteins involved in the IFN axis (CXCL9, CXCL11), known psoriasis-related proteins (peptidase inhibitor-3/elafin, SELP), B-cell related protein (IGLC2), neutrophil-related markers (LCN2, SERPINA5, SLAMF1), and markers associated with general inflammation (HGF, HO-1) (Figure 4c).

G-CSF or CSF3 is a major hematopoietic cytokine regulating granulopoiesis and is involved in inducing both granulocyte production and release from the bone marrow (Furze and Rankin, 2008; Semerad et al., 2002). Given the strong correlation between neutrophil-related biomarkers in the serum and skin and the neutrophilic signature associated with HS overall, we asked whether CSF3 is a possible driver of increased neutrophil activity. The mRNA levels of *CSF3* were elevated in the LS and PL skin of patients with HS compared with the skin from healthy volunteers. Therefore, we asked whether there was a correlation between CFS3 levels in LS skin and neutrophil-related biomarkers in the serum (Figure 4d). Indeed, many of the neutrophil-related markers correlated with increased expression of *CSF3* mRNA in the skin, suggesting that the active inflammatory lesion may be driving the recruitment of neutrophils and thus increasing the expression of neutrophil-related proteins in the serum (Figure 4d).

Levels of neutrophil-related proteins in the serum correlate with HS clinical activity

We then asked whether clinical characteristics correlated with LCN2 and IL-17A protein levels in the serum (Figure 5). Given that only patients with advanced HS (Hurley stages II and III) were included in this study, we could not correlate the serum levels of LCN2 and IL-17A with Hurley stage. However, patients with Hurley stage III were more likely to present with draining tunnels ($P = 0.0091$) and thus were more likely to have neutrophilic inflammation in the serum, consistent with the analysis in Figure 3d. Unlike the

International Hidradenitis Suppurativa Severity Score System criteria, Hurley staging does not take into account the presence of nodules, abscesses and draining tunnels in a weighted approach. International Hidradenitis Suppurativa Severity Score System, which assigns weighted points to the number of nodules, abscesses, and draining tunnels or fistulae, correlated the most with serum protein levels of LCN2 and IL-17A, suggesting its utility as a tool to quantify disease activity and clinical response in HS. Therefore, our data suggest that the International Hidradenitis Suppurativa Severity Score System score may be more representative of disease activity than Hurley staging. Furthermore, International Hidradenitis Suppurativa Severity Score System scores also correlated with other markers of general inflammation (TNF-α, IL-6) and biomarkers of cardiovascular risk (ST2, HGF, TIE2). This may suggest that patients with more severe HS are at an increased risk of cardiovascular disease.

DISCUSSION

This study presents a large-scale proteomic analysis of serum from patients with moderate-to-severe HS. Consistent with previous reports in the skin, we identified an elevation of IL-17A in HS serum (Kelly et al., 2015; Lima et al., 2016; Navrazhina et al., 2020). We demonstrate systemic neutrophilic inflammation in HS, consistent with elevated absolute neutrophil counts in the blood (Supplementary Table S1). Unbiased analysis of samples demonstrated clustering of HS on the basis of high and low LCN2 expression in the serum, which corresponded with histologically confirmed presence or absence of epithelialized dermal tunnels, respectively. This is consistent with neutrophils and keratinocytes (likely from the epithelized tunnels) being the source of LCN2 elevation (Wolk et al., 2017). There was a significant serum–skin correlation of neutrophilic markers present in the PL skin, suggesting that there is an ongoing systemic inflammation even in healthy-appearing skin. Consistent with this, smaller-scale studies have shown that there is an upregulation of proinflammatory pathways even in healthy-appearing unaffected skin, further giving credence to the concept of HS as a systemic dermatosis (Navrazhina et al., 2020; Sanchez et al., 2019; van der Zee et al., 2011). Consistent with our work, previous ELISA-based analysis of HS serum has demonstrated dysregulation in pathways involving general inflammation (Blok et al., 2016), neutrophil activation (Wolk et al., 2017), complement pathway (Hoffman et al., 2018), and antibody formation (Assan et al., 2020). We identified CSF3 in the skin as a potential regulator of neutrophilic inflammation in the serum. Taken together, our data suggest that HS has significant clinical and molecular heterogeneity, demonstrating that HS patients with dermal tunnels have a different proteomic profile.

HS is a heterogeneous disease in its clinical presentation; however, it is unknown whether different morphological structures manifest in unique inflammatory signatures in HS skin and serum. We present a large-scale proteomic analysis demonstrating an unbiased clustering of HS disease into distinct subgroups. HS had fewer DEPs than psoriasis and AD, which could reflect a smaller body surface area affected, the compartmentalization of immune response in HS and the heterogeneity of our patient cohort likely affecting the number of statistically significant DEPs. When subdivided by the presence of tunnels, there was a higher number of DEPs than when examining the entire heterogeneous group. We demonstrate an interesting association between a morphological structure in the skin (tunnels) and serum biomarkers. Patients with draining tunnels had significantly higher levels of neutrophil-related proteins in the serum (IL-17A, MPO, LCN2, CXCL8, EN-RAGE, DEFA1, matrix metalloproteinase 9) than those with nondraining tunnels. This is consistent with

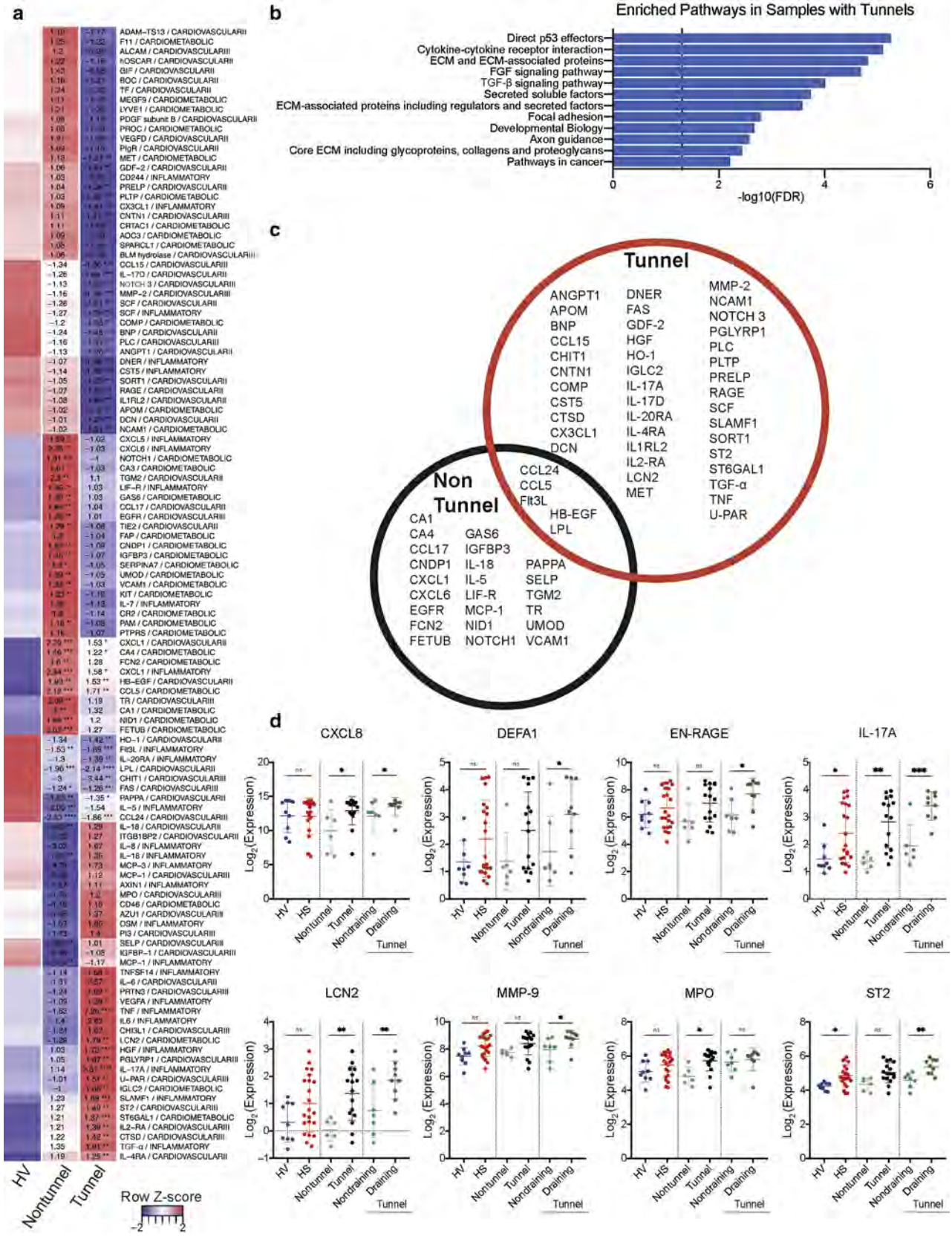

Figure 3. HS patients with tunnels have a different serum proteome profile compared to patients without tunnels. (**a**) Heatmap of all differentially expressed proteins (abs [FCH] ≥1.2, and $P \leq 0.05$) between HVs and HS patients without tunnels, HVs and HS patients with tunnels, or between HS patients with without tunnels. FCHs relative to those of HVs are shown; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. (**b**) Enriched biological processes in the serum of HS patients with tunnels by canonical, KEGG, Reactome and BioCarta pathways using the XGR tool. Vertical line shows FDR = 0.05. (**c**) Venn Diagram of the differentially expressed proteins in HS patients with and without tunnels relative to HVs. (**d**) Olink expression of serum protein levels shown in Log_2(Expression) for neutrophil

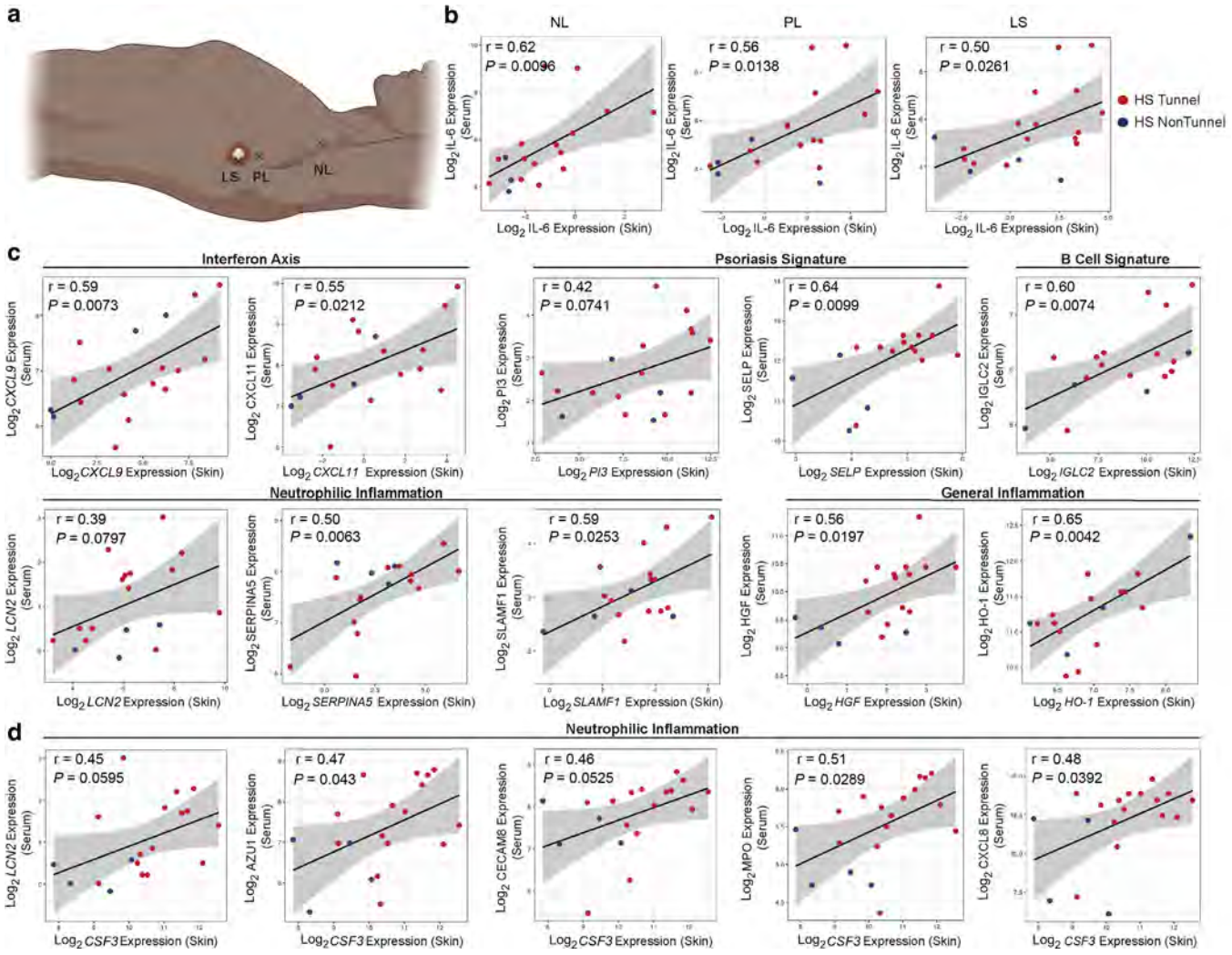

Figure 4. There is a significant serum–skin correlation in HS. (**a**) LS skin was biopsied at an edge of an active inflammatory lesion. PL and NL skin biopsies were taken from healthy-appearing skin 2 cm and 10 cm from the edge of the active inflammatory lesion, respectively, and were biopsied on the same anatomical area as the LS biopsy. (**b**) Correlation plots of IL-6 protein serum levels and the *IL-6* mRNA levels in the LS, PL, and NL skin; scatterplots are shown with estimated linear regression and 95% confidence interval; r is Pearson correlation. (**c**) Serum–skin correlation of serum protein levels (Log_2 Olink expression) with their corresponding mRNA levels in the PL skin (Log_2 mRNA expression). (**d**) Serum–skin correlation between mRNA levels of *CSF3* in the LS skin and the levels of neutrophil-related proteins in the serum. HS, hidradenitis suppurativa; LS, lesional; NL, nonlesional; PL, perilesional.

the pus in draining tunnels being neutrophil mediated. Interestingly, HS patients with tunnels demonstrated an enrichment of pathways related to ECM remodeling and developmental biology. These signatures may explain the development of dermal tunnels and shift the paradigm from tunnels being an end-stage fibrotic feature of the disease to an active inflammatory structure. LCN2 is a protein secreted by granulocytes, neutrophils, and keratinocytes. TNF-α is a potent inducer of LCN2 in granulocytes, whereas TNF-α and IL-17 have been shown to induce LCN2 production in keratinocytes (Wolk et al., 2017). Consistent with this, patients with tunnels had increased levels of IL-17A and TNF-α in the serum, which provides the direct mechanistic link for the increased levels of LCN2 in patients with tunnels. Whereas some studies have shown that LCN2 is associated with obesity (Koiou et al., 2012; Mosialou et al., 2020; Wang et al., 2007) and could provide some cardiometabolic protection (Mosialou et al., 2020), a study of patients with psoriasis did not find a correlation between BMI and LCN2 levels but did report an elevation of LCN2 in the serum of psoriasis patients compared with healthy controls (Kamata et al., 2012). Similarly, we did not find a significant difference in BMI between LCN2-low and LCN2-high patients in our cohort ($P = 0.34$). In our study, LCN2 levels in the serum are correlated with neutrophilic markers and the number of tunnels, suggesting that the strong LCN2 signature associated with the disease activity and that the presence of tunnels may supersede any differences related to the BMI. We believe that

and cardiovascular risk–related proteins in the serum of HVs and HS patients, HS patients with and without tunnels, and HS patients with draining and nondraining tunnels. Each dot represents an individual sample. abs, absolute value; ECM, extracellular matrix; FCH, fold change; FDR, false discovery rate; HS, hidradenitis suppurativa; HV, healthy volunteer; KEGG, Kyoto Encyclopedia of Genes and Genomes; MMP, matrix metalloproteinase; ns, nonsignificant; XGR, eXploring Genomic Relations.

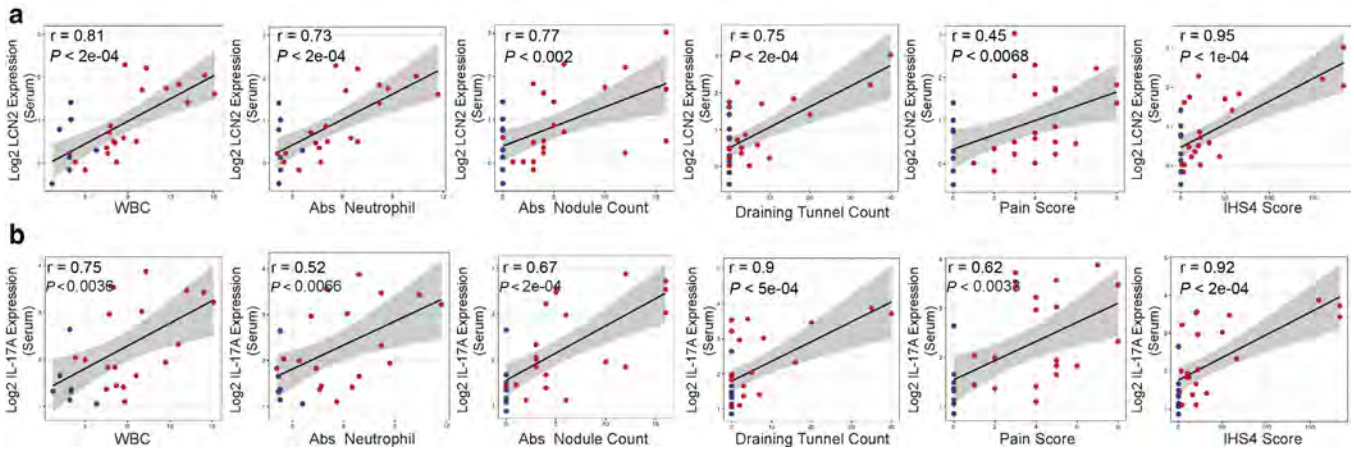

Figure 5. Serum protein correlations with clinical markers and skin disease severity. Correlation of WBC count, Abs neutrophil count, Abs nodule count (defined as the sum of nodules and abscesses), draining tunnel count, pain score, and IHS4 scores with (**a**) LCN2 and (**b**) IL-17A protein levels in the serum. Scatterplots are shown with estimated linear regression and 95% confidence interval; r is Pearson correlation. Blue dots represent healthy control samples, and red dots represent HS samples. Abs, absolute; HS, hidradenitis suppurativa; IHS4, International Hidradenitis Suppurativa Severity Score System; WBC, white blood cell.

the elevated LCN2 levels were likely derived from the high inflammatory burden of the disease rather than BMI. This highlights the role of tunnels in disease activity. Patients with draining tunnels had significantly higher levels of ST2 protein than those with nondraining tunnels, suggesting that the inflammation extends beyond the skin and may potentially affect cardiovascular health.

Furthermore, the presence of tunnels influences the time it takes to achieve Hidradenitis Suppurativa Clinical Response in the PIONEER study of adalimumab (Frew et al., 2020, 2021). Our data provide the molecular mechanism for why patients with tunnels have a different disease activity and treatment response (Frew et al., 2021). Differences in biomarkers between tunnels and nontunnel samples may define disease endotypes that could impact therapeutic choices. For example, we found higher levels of TNF in tunnel-positive individuals, and it has been shown that the presence of tunnels increases the time to the clinical response to adalimumab (Frew et al., 2021). We speculate that the high levels of TNF could require the use of high-dose TNF antibodies that have been shown to be effective in severe cases (Ghias et al., 2020). Our study provides evidence of how morphological structures could influence the molecular profile of patients with HS. Identifying subjects with HS (potentially on the basis of the presence or absence of tunnels) can identify not just novel biomarkers specific to each disease subset but may also potentially identify effective treatment unique to each subgroup with HS.

Our findings demonstrate that the skin may be a driver of the neutrophilic inflammation in the serum as we observed a proportional correlation between *CSF3* mRNA in the skin and neutrophil-related proteins in the serum. We had previously reported an increasing gradient of IL-17 from nonlesional to LS skin (Navrazhina et al., 2020). IL-17 has been shown to stimulate granulopoiesis by inducing G-CSF (Hirai et al., 2012; Schwarzenberger et al., 2000, 1998; Xu and Cao, 2010). It is plausible that IL-17, TNF-α, and IL-6 in HS skin may stimulate G-CSF production by fibroblasts, monocytes, and endothelial cells, which in turn stimulates the release of neutrophils from the bone marrow (Kaushansky, 2006; Xu and Cao, 2010). Potentially, G-CSF could affect cutaneous characteristics as several individual case reports have reported psoriasiform cutaneous eruption in patients receiving G-CSF (Cho et al., 1998; Jang et al., 2017; Kavanaugh, 1996; Mössner et al., 2004).

Acute and chronic pain contributes significantly to the reduced QOL in patients with HS (Savage et al., 2020). In this study, we explored the correlation between clinical parameters and the levels of neutrophilic proteins in the serum. Of particular interest is the correlation between LCN2 and IL-17A and the reported pain levels in HS. The mechanisms of pain levels in HS have not been elucidated. Reports have suggested that neutrophil chemoattractant leukotriene B_4 as well as the migration cascade of neutrophils themselves can lead to hyperalgesia and mechanical hypernociception (Cunha et al., 2008; Levine et al., 1984). Consistent with this, animal studies have shown that both LCN2 and IL-17A are involved in mechanical hyperalgesia (Ebbinghaus et al., 2017; Jeon et al., 2013). Our data provide a plausible mechanism of how high levels of neutrophilic proteins may contribute to the pain burden in HS.

A strength of our study is that we assessed a large panel of known biomarkers in an unbiased approach. Consistent with our data, previous studies of HS serum demonstrated elevated levels of IL-17A, TNF-α, LCN2, and IL-6 (Jiménez-Gallo et al., 2017; Matusiak et al., 2017, 2009; Wolk et al., 2017). However, the clinical disease heterogeneity in HS complicates the identification of biomarkers. All of the patients in our study were either untreated or had undergone a washout period, therefore eliminating these confounders in the analysis. Furthermore, given that changes in proteomic profiles are related to changes in BMI and fat distribution, we utilized BMI-matched healthy controls (Lind et al., 2020). Importantly, rather than focusing on known disease-associated cytokines, we sought to determine previously unreported disease-associated biomarkers through the use of a large biomarker panel and a hypothesis-free approach.

The limitations of our work include a modest sample size (although comparable with those of other cohorts studied [Blok et al., 2016; Brunner et al., 2019; Wolk et al., 2017]), the use of controls who were older than the HS cohort, and the relative limitation of the Olink platform where analysis is restricted to pregrouped biomarker subsets. Future larger-scale studies are warranted to identify how other HS manifestations (abscesses vs. nodule, draining vs. nondraining tunnel) impact serum proteome. Additionally, our analysis is limited to patients with moderate and severe HS, and it would be desirable to study patients with new onset of HS or patients with mild HS in future studies. If serum biomarkers are also elevated in the early-stage disease, these biomarkers may facilitate diagnosis and decrease the 5−14 year diagnostic delay experienced by patients with HS (Jemec and Kimball, 2015). Given the cyclical nature of HS severity, marked by debilitating flare ups and a remitting course, serum biomarkers become particularly crucial to assess disease activity and accurately diagnose as well as measure therapeutic response.

In conclusion, we demonstrate that HS is a systemic, inflammatory condition associated with neutrophil-rich signature in the serum, with a significant serum–skin correlation of neutrophilic activity. We identify a highly-inflammatory HS subgroup corresponding with increased LCN2 protein levels in the serum and histologically confirmed tunnels in the skin.

MATERIALS AND METHODS

Patients

The study was approved by the Institutional Review Board of The Rockefeller University (New York, NY), and written informed consent was obtained. In total, 22 patients with Hurley stage II (n = 15) and stage III (n = 7) and nine BMI-matched healthy controls were included in this study (Supplementary Table S1). Exclusion criteria included being diagnosed with HIV and hepatitis B or C, being currently pregnant, or breastfeeding. Patients were required to undergo a washout period of five half-lives from previous systemic treatments, including all oral antibiotics, retinoid, and biologic therapies.

Serum protein quantification

Samples were centrifuged after collection, and serum was stored at −80 °C. Samples were analyzed using the proteomic Olink Proseek multiplex assay. Serum (10 μl) was used for proximity extension assay, which uses a real-time PCR to detect oligonucleotide-labeled antibody probe pairs to individual targets, as previously described (Assarsson et al., 2014; Bettoli et al., 2016). Samples were assessed using the Olink Inflammation (92 analytes), cardiovascular II (92 analytes) and cardiovascular III (92 analytes), and cardiometabolic panel (92 analytes). All of the samples met the quality control (QC) for the Olink panels with the exception of one healthy control sample that did not meet the QC for cardiovascular II and inflammation panels and was therefore excluded from the analysis within these two panels. Only samples that had detected the expression of all the proteins in the panels were included in the heatmaps.

Skin mRNA quantification

RNA from frozen skin biopsies was isolated using miRNeasy Mini Kit (Qiagen, Hilden, Germany), and DNA was removed using on-column DNase digestion from the RNase-free DNase Set (Qiagen). RNA sequencing was performed using NovaSeq 6000 (Illumina, San Diego, CA), and analysis was conducted as previously described (Suárez-Fariñas et al., 2015; Visvanathan et al., 2019). RT-PCR was used to assess *CSF3* expression in the LS skin, and expression of *CSF3* mRNA in the skin was normalized to the house-keeping gene *hARP* as previously described (Navrazhina et al., 2020). Probes used were TaqMan Gene Expression Assay CSF3 (Hs00738432_g1) and hARP (AID1UP5) from Thermo Fisher Scientific (Waltham, MA).

Statistical analysis

Statistical analysis was performed in R language (R-project.org, R Foundation, Vienna, Austria) using publicly available Bioconductor Project packages (www.bioconductor.org; Bioconductor Core Team, Buffalo, NY). QC of Olink data was accomplished using Olink's standard QC pipeline (Lind et al., 2015). One healthy control sample did not pass the QC for cardiovascular II and inflammatory panel quantification and therefore was excluded from the analysis in both panels. The Olink platform presents data in Normalized Protein eXpression arbitrary Log_2 scaled units. Protein expression profiles were modeled with linear models using the limma framework as previously described (Brunner et al., 2017). This model considers disease state and tunnel status as fixed factors, whereas random effect related to the subjects was included in the model (Brunner et al., 2017; He et al., 2020). The least squared means and comparisons for protein profiles among the different groups were estimated, and hypothesis testing was performed under the general framework for linear models in the limma package. A sensitivity analysis for the smoking status was implemented, demonstrating no imputation-related departures from conclusions reached. DEPs were defined as those with absolute value of (fold change) ≥ 1.2 and $P \leq 0.05$, consistent with previous studies (Brunner et al., 2017; He et al., 2020) Correlation between mRNA levels in the skin, protein expression in the serum, and clinical parameters were evaluated using Pearson correlations on log_2-transformed expression values.

Pathway analysis

Gene set enrichment analysis was performed using the eXploring Genomic Relations (accessed 11/15/2020) (Fang et al., 2016) for biological processes pathways, including Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al., 2010), BioCarta (Croft et al., 2014), Reactome (Croft et al., 2014), and Gene Ontology (The Gene Ontology Consortium, 2019). False discovery rate cutoff at 0.05 was used to identify significant enrichment. Statistical overrepresentation test was performed using Protein Annotation Through Evolutionary Relationship Gene Ontology cellular component complete analysis tool (Mi et al., 2019). Significance was defined as a false discovery rate < 0.05.

Data availability statement

The datasets related to this article can be found at https://doi.org/10.17632/jk7bb355tr and https://data.mendeley.com/datasets/jk7bb355tr/2 hosted at Mendeley. All other supporting data are available on written request to the corresponding author.

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CONFLICT OF INTEREST

JGK has received research support (grants paid to institution) from AbbVie, Amgen, Bristol Myers Squibb, Boehringer Ingelheim, EMD Serono,

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AUTHOR CONTRIBUTIONS

Conceptualization: KN, JWF, JGK; Data Curation: KN, SG; Formal Analysis: SG, KN; Funding Acquisition: JWF, JGK; Investigation: KN, JG, DG, JWF; Methodology: JG, KN, JWF, DG; Supervision: JGK; Writing - Original Draft Preparation: KN, JWF; Writing - Review and Editing: JGK

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.02.742.

REFERENCES

Assan F, Gottlieb J, Tubach F, Lebbah S, Guigue N, Hickman G, et al. Anti-Saccharomyces cerevisiae IgG and IgA antibodies are associated with systemic inflammation and advanced disease in hidradenitis suppurativa. J Allergy Clin Immunol 2020;146:452–5.e5.

Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 2014;9:e95192.

Bell EJ, Decker PA, Tsai MY, Pankow JS, Hanson NQ, Wassel CL, et al. Hepatocyte growth factor is associated with progression of atherosclerosis: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2018;272:162–7.

Bettoli V, Join-Lambert O, Nassif A. Antibiotic treatment of hidradenitis suppurativa. Dermatol Clin 2016;34:81–9.

Blok JL, Li K, Brodmerkel C, Horvátovich P, Jonkman MF, Horváth B. Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. Br J Dermatol 2016;174:839–46.

Brunner PM, He H, Pavel AB, Czarnowicki T, Lefferdink R, Erickson T, et al. The blood proteomic signature of early-onset pediatric atopic dermatitis shows systemic inflammation and is distinct from adult long-standing disease. J Am Acad Dermatol 2019;81:510–9.

Brunner PM, Suárez-Fariñas M, He H, Malik K, Wen HC, Gonzalez J, et al. The atopic dermatitis blood signature is characterized by increases in inflammatory and cardiovascular risk proteins. Sci Rep 2017;7:8707.

Byrd AS, Carmona-Rivera C, O'Neil LJ, Carlucci PM, Cisar C, Rosenberg AZ, et al. Neutrophil extracellular traps, B cells, and type I interferons contribute to immune dysregulation in hidradenitis suppurativa. Sci Transl Med 2019;11:eaav5908.

Byrd AS, Kerns ML, Williams DW, Zarif JC, Rosenberg AZ, Delsante M, et al. Collagen deposition in chronic hidradenitis suppurativa: potential role for $CD163^{+}$ macrophages. Br J Dermatol 2018;179:792–4.

Chassaing B, Srinivasan G, Delgado MA, Young AN, Gewirtz AT, Vijay-Kumar M. Fecal lipocalin 2, a sensitive and broadly dynamic non-invasive biomarker for intestinal inflammation. PLoS One 2012;7:e44328.

Cho SG, Park YM, Moon H, Kim KM, Bae SS, Kim GB, et al. Psoriasiform eruption triggered by recombinant granulocyte-macrophage colony stimulating factor (rGM-CSF) and exacerbated by granulocyte colony stimulating factor (rG-CSF) in a patient with breast cancer. J Korean Med Sci 1998;13:685–8.

Croft D, Mundo AF, Haw R, Milacic M, Weiser J, Wu G, et al. The Reactome pathway knowledgebase. Nucleic Acids Res 2014;42:D472–7.

Cunha TM, Verri WA Jr, Schivo IR, Napimoga MH, Parada CA, Poole S, et al. Crucial role of neutrophils in the development of mechanical inflammatory hypernociception. J Leukoc Biol 2008;83:824–32.

Ebbinghaus M, Natura G, Segond von Banchet G, Hensellek S, Böttcher M, Hoffmann B, et al. Interleukin-17A is involved in mechanical hyperalgesia but not in the severity of murine antigen-induced arthritis. Sci Rep 2017;7:10334.

Fang H, Knezevic B, Burnham KL, Knight JC. XGR software for enhanced interpretation of genomic summary data, illustrated by application to immunological traits. Genome Med 2016;8:129.

Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, et al. Clinical response rates, placebo response rates, and significantly associated covariates are dependent on choice of outcome measure in hidradenitis suppurativa: a post hoc analysis of PIONEER 1 and 2 individual patient data. J Am Acad Dermatol 2020;82:1150–7.

Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, et al. Dermal tunnels influence time to clinical response and family history influences time to loss of clinical response in patients with hidradenitis suppurativa treated with adalimumab. Clin Exp Dermatol 2021;46:306–13.

Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR, Kirby JS, et al. Defining lesional, perilesional and unaffected skin in hidradenitis suppurativa: proposed recommendations for clinical trials and translational research studies. Br J Dermatol 2019;181:1339–41.

Furze RC, Rankin SM. Neutrophil mobilization and clearance in the bone marrow. Immunology 2008;125:281–8.

Ghias MH, Johnston AD, Kutner AJ, Micheletti RG, Hosgood HD, Cohen SR. High-dose, high-frequency infliximab: a novel treatment paradigm for hidradenitis suppurativa. J Am Acad Dermatol 2020;82:1094–101.

Glickman JW, Dubin C, Renert-Yuval Y, Dahabreh D, Kimmel GW, Auyeung K, et al. Cross-sectional study of blood biomarkers of patients with moderate to severe alopecia areata reveals systemic immune and cardiovascular biomarker dysregulation. J Am Acad Dermatol 2021;84:370–80.

Gudjonsson JE, Tsoi LC, Ma F, Billi AC, van Straalen KR, Vossen ARJV, et al. Contribution of plasma cells and B cells to hidradenitis suppurativa pathogenesis. JCI Insight 2020;5:e139930.

He H, Del Duca E, Diaz A, Kim HJ, Gay-Mimbrera J, Zhang N, et al. Mild atopic dermatitis lacks systemic inflammation and shows reduced nonlesional skin abnormalities [e-pub ahead of print]. J Allergy Clin Immunol 2020. https://doi.org/10.1016/j.jaci.2020.08.041 (accessed October 1, 2020).

Hirai Y, Iyoda M, Shibata T, Kuno Y, Kawaguchi M, Hizawa N, et al. IL-17A stimulates granulocyte colony-stimulating factor production via ERK1/2 but not p38 or JNK in human renal proximal tubular epithelial cells. Am J Physiol Renal Physiol 2012;302:F244–50.

Hoffman LK, Tomalin LE, Schultz G, Howell MD, Anandasabapathy N, Alavi A, et al. Integrating the skin and blood transcriptomes and serum proteome in hidradenitis suppurativa reveals complement dysregulation and a plasma cell signature. PLoS One 2018;13:e0203672.

Jang MS, Park JB, Kim JH, Yang MH, Lee KH, Han SH, et al. Granulocyte colony-stimulating factor-induced psoriasiform dermatitis improved by narrowband ultraviolet B. Ann Dermatol 2017;29:232–3.

Jemec GB, Heidenheim M, Nielsen NH. The prevalence of hidradenitis suppurativa and its potential precursor lesions. J Am Acad Dermatol 1996;35:191–4.

Jemec GB, Kimball AB. Hidradenitis suppurativa: epidemiology and scope of the problem. J Am Acad Dermatol 2015;73(Suppl. 1):S4–7.

Jeon S, Jha MK, Ock J, Seo J, Jin M, Cho H, et al. Role of lipocalin-2-chemokine axis in the development of neuropathic pain following peripheral nerve injury. J Biol Chem 2013;288:24116–27.

Jiménez-Gallo D, de la Varga-Martínez R, Ossorio-García L, Albarrán-Planelles C, Rodríguez C, Linares-Barrios M. The clinical significance of increased serum proinflammatory cytokines, C-reactive protein, and erythrocyte sedimentation rate in patients with hidradenitis suppurativa. Mediators Inflamm 2017;2017:2450401.

Kamata M, Tada Y, Tatsuta A, Kawashima T, Shibata S, Mitsui H, et al. Serum lipocalin-2 levels are increased in patients with psoriasis. Clin Exp Dermatol 2012;37:296–9.

Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M. KEGG for representation and analysis of molecular networks involving diseases and drugs. Nucleic Acids Res 2010;38:D355–60.

Kaushansky K. Lineage-specific hematopoietic growth factors. N Engl J Med 2006;354:2034–45.

Kavanaugh A. Flare of psoriasis and psoriatic arthritis following treatment with granulocyte colony-stimulating factor. Am J Med 1996;101:567–8.

Kelly G, Hughes R, McGarry T, van den Born M, Adamzik K, Fitzgerald R, et al. Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. Br J Dermatol 2015;173:1431–9.

Kim J, Tomalin L, Lee J, Fitz LJ, Berstein G, Correa-da Rosa J, et al. Reduction of inflammatory and cardiovascular proteins in the blood of patients with psoriasis: differential responses between tofacitinib and etanercept after 4 weeks of treatment. J Invest Dermatol 2018;138:273–81.

Koiou E, Tziomalos K, Katsikis I, Kandaraki EA, Kalaitzakis E, Delkos D, et al. Weight loss significantly reduces serum lipocalin-2 levels in overweight and obese women with polycystic ovary syndrome. Gynecol Endocrinol 2012;28:20–4.

Kurek A, Johanne Peters EM, Sabat R, Sterry W, Schneider-Burrus S. Depression is a frequent co-morbidity in patients with acne inversa. J Dtsch Dermatol Ges 2013;11:743–50.

Levine JD, Lau W, Kwiat G, Goetzl EJ. Leukotriene B4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. Science 1984;225:743–5.

Lima AL, Karl I, Giner T, Poppe H, Schmidt M, Presser D, et al. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. Br J Dermatol 2016;174:514–21.

Lind L, Ärnlöv J, Lindahl B, Siegbahn A, Sundström J, Ingelsson E. Use of a proximity extension assay proteomics chip to discover new biomarkers for human atherosclerosis. Atherosclerosis 2015;242:205–10.

Lind L, Figarska S, Sundström J, Fall T, Ärnlöv J, Ingelsson E. Changes in proteomic profiles are related to changes in BMI and fat distribution during 10 years of aging. Obesity (Silver Spring) 2020;28:178–86.

Lowe MM, Naik HB, Clancy S, Pauli M, Smith KM, Bi Y, et al. Immunopathogenesis of hidradenitis suppurativa and response to anti-TNF-α therapy. JCI Insight 2020;5:e139932.

Matusiak L, Bieniek A, Szepietowski JC. Increased serum tumour necrosis factor-alpha in hidradenitis suppurativa patients: is there a basis for treatment with anti-tumour necrosis factor-alpha agents? Acta Derm Venereol 2009;89:601–3.

Matusiak L, Bieniek A, Szepietowski JC. Psychophysical aspects of hidradenitis suppurativa. Acta Derm Venereol 2010;90:264–8.

Matusiak Ł, Szczęch J, Bieniek A, Nowicka-Suszko D, Szepietowski JC. Increased interleukin (IL)-17 serum levels in patients with hidradenitis suppurativa: implications for treatment with anti-IL-17 agents. J Am Acad Dermatol 2017;76:670–5.

Mi H, Muruganujan A, Huang X, Ebert D, Mills C, Guo X, et al. Protocol update for large-scale genome and gene function analysis with the PANTHER classification system (v.14.0). Nat Protoc 2019;14:703–21.

Miller IM, Ellervik C, Vinding GR, Zarchi K, Ibler KS, Knudsen KM, et al. Association of metabolic syndrome and hidradenitis suppurativa. JAMA Dermatol 2014;150:1273–80.

Mosialou I, Shikhel S, Luo N, Petropoulou PI, Panitsas K, Bisikirska B, et al. Lipocalin-2 counteracts metabolic dysregulation in obesity and diabetes. J Exp Med 2020;217:e20191261.

Mössner R, Beckmann I, Hallermann C, Neumann C, Reich K. Granulocyte colony-stimulating-factor-induced psoriasiform dermatitis resembles psoriasis with regard to abnormal cytokine expression and epidermal activation. Exp Dermatol 2004;13:340–6.

Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. Br J Dermatol 2020;182:1045–7.

Reddy S, Strunk A, Jemec GBE, Garg A. Incidence of myocardial infarction and cerebrovascular accident in patients with hidradenitis suppurativa. JAMA Dermatol 2020;156:65–71.

Sabat R, Chanwangpong A, Schneider-Burrus S, Metternich D, Kokolakis G, Kurek A, et al. Increased prevalence of metabolic syndrome in patients with acne inversa. PLoS One 2012;7:e31810.

Sabat R, Jemec GBE, Matusiak Ł, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. Nat Rev Dis Primers 2020;6:18.

Sanchez J, Le Jan S, Muller C, François C, Renard Y, Durlach A, et al. Matrix remodelling and MMP expression/activation are associated with hidradenitis suppurativa skin inflammation. Exp Dermatol 2019;28:593–600.

Savage KT, Singh V, Patel ZS, Yannuzzi CA, McKenzie-Brown AM, Lowes MA, et al. Pain management in hidradenitis suppurativa and a proposed treatment algorithm [e-pub ahead of print]. J Am Acad Dermatol 2020. https://doi.org/10.1016/j.jaad.2020.09.039 (accessed October 1, 2020).

Schroll A, Eller K, Feistritzer C, Nairz M, Sonnweber T, Moser PA, et al. Lipocalin-2 ameliorates granulocyte functionality. Eur J Immunol 2012;42:3346–57.

Schwarzenberger P, Huang W, Ye P, Oliver P, Manuel M, Zhang Z, et al. Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. J Immunol 2000;164:4783–9.

Schwarzenberger P, La Russa V, Miller A, Ye P, Huang W, Zieske A, et al. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. J Immunol 1998;161:6383–9.

Semerad CL, Liu F, Gregory AD, Stumpf K, Link DC. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. Immunity 2002;17:413–23.

Suárez-Fariñas M, Ungar B, Correa da Rosa J, Ewald DA, Rozenblit M, Gonzalez J, et al. RNA sequencing atopic dermatitis transcriptome profiling provides insights into novel disease mechanisms with potential therapeutic implications. J Allergy Clin Immunol 2015;135:1218–27.

The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Res 2019;47:D330–8.

Thomi R, Schlapbach C, Yawalkar N, Simon D, Yerly D, Hunger RE. Elevated levels of the antimicrobial peptide LL-37 in hidradenitis suppurativa are associated with a Th1/Th17 immune response. Exp Dermatol 2018;27:172–7.

Thorsvik S, Damås JK, Granlund AV, Flo TH, Bergh K, Østvik AE, et al. Fecal neutrophil gelatinase-associated lipocalin as a biomarker for inflammatory bowel disease. J Gastroenterol Hepatol 2017;32:128–35.

van der Zee HH, de Ruiter L, van den Broecke DG, Dik WA, Laman JD, Prens EP. Elevated levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-α and IL-1β. Br J Dermatol 2011;164:1292–8.

van der Zee HH, de Winter K, van der Woude CJ, Prens EP. The prevalence of hidradenitis suppurativa in 1093 patients with inflammatory bowel disease. Br J Dermatol 2014;171:673–5.

Villacorta H, Maisel AS. Soluble ST2 testing: a promising biomarker in the management of heart failure. Arq Bras Cardiol 2016;106:145–52.

Visvanathan S, Baum P, Vinisko R, Schmid R, Flack M, Lalovic B, et al. Psoriatic skin molecular and histopathologic profiles after treatment with risankizumab versus ustekinumab. J Allergy Clin Immunol 2019;143:2158–69.

Vossen ARJV, van der Zee HH, Tsoi LC, Xing X, Devalaraja M, Gudjonsson JE, et al. Novel cytokine and chemokine markers of hidradenitis suppurativa reflect chronic inflammation and itch. Allergy 2019;74:631–4.

Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, et al. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem 2007;53:34–41.

Wolk K, Frambach Y, Jacobi A, Wilsmann-Theis D, Phillipp S, Witte-Händel E, et al. Increased levels of lipocalin 2 in palmoplantar pustular psoriasis. J Dermatol Sci 2018;90:68–74.

Wolk K, Warszawska K, Hoeflich C, Witte E, Schneider-Burrus S, Witte K, et al. Deficiency of IL-22 contributes to a chronic inflammatory disease: pathogenetic mechanisms in acne inversa. J Immunol 2011;186:1228–39.

Wolk K, Wenzel J, Tsaousi A, Witte-Händel E, Babel N, Zelenak C, et al. Lipocalin-2 is expressed by activated granulocytes and keratinocytes in affected skin and reflects disease activity in acne inversa/hidradenitis suppurativa. Br J Dermatol 2017;177:1385–93.

Xu S, Cao X. Interleukin-17 and its expanding biological functions. Cell Mol Immunol 2010;7:164–74.

4.3: The Role of Genetics in the Pathogenesis of HS:

Manuscript	Manuscript Reference
3-1	Frew JW, Vekic DA, Woods J, Cains GD (2017) "A Systematic Review and Critical Evaluation of Reported Pathogenic Sequence Variants in Hidradenitis Suppurativa" British Journal of Dermatology 2017; DOI:10.1111/bjd.15441
3-2	Frew JW, Hawkes JE, Sullivan-Whalen M, Gilleaudeau P, Krueger JG "Inter-Relater Reliability of Phenotypes, and Exploratory Genotype-Phenotype Analysis in Inherited Hidradenitis Suppurativa" Br J Dermatol 2019; 2019 Jan 28. doi: 10.1111/bjd.1769
3-3	Frew JW, Navrazhina K, In-Silico Analysis of Gamma-Secretase-Complex Mutations in Hidradenitis Suppurativa Demonstrates Disease-Specific Substrate Recognition and Cleavage Alterations Front Med 2019; DOI: 10.3389/fmed.2019.00206
3-4	Frew JW "We Need to Talk About Notch: Notch Dysregulation as an Epiphenomenon in Inflammatory Skin Disease" (2018) Br J Dermatol 2019 Feb;180(2):431-432
3-5	Frew JW Navrazhina K No Evidence that Impaired Notch Signaling Differentiates Hidradenitis Suppurativa from other Inflammatory Skin Diseases Br J Dermatol 2020;182(4):1042-1043

Table 4.3.1: List of publications included in this chapter pertaining to the role of genetics in HS.

Hidradenitis Suppurativa has been identified as a condition with strong familial predilection for close to 40 years[68,69], however it was only in 2010 when gene linkage analysis in an East-Asian kindred identified an association with the Nicastrin component of the Gamma Secretase Complex[70]. Since that time, further case reports and kindred studies have identified multiple polymorphisms in various aspects of the Gamma Secretase Complex associated with HS[71], with subsequent data suggesting a downstream downregulation of Notch activity[72]. This has led to the paradigm of familial HS being a monogenic disorder associated with polymorphisms in the GSC[70-72]. To

date, genetic data has only been identified in disparate kindred from various population groups. Data from large scale genome wide association studies in HS are not yet available. There is disparity between genetic association reports from various geographical subpopulations. Whilst Gamma Secretase Complex associated gene polymorphisms are common in East-Asian kindred, similar rates were not replicated in European Caucasian cohorts[73].

This chapter presents five publications (Table 4-3-1) which critically evaluate the types of genetic polymorphisms identified in HS and their impact upon GSC function, and then secondly provide novel in-silico based data to propose and support an alternative hypothesis to the Notch-centric paradigm. The first publication (3.1) is the first systematic review of all reported polymorphisms in familial HS. Polymorphisms were identified and then structurally mapped to the known protein structure of the individual components of the Gamma Secretase Complex structure. Through these methods, it was identified that many polymorphisms in Nicastrin (the most common component of the GCS affected in HS) impacted the protein structure of the extracellular component of the GCS. This is contrast to the hypothesized mechanism of GSC polymorphisms in HS in which complete loss of a GSC component (such as Nicastrin) would lead to decreased intracellular signalling. However, structural alterations of the extracellular component of Nicastrin has been demonstrated to lead to over activation of intracellular pathways[74] in Alzheimer's disease- familial forms of which also impact aspects of the GCS[74].

The second publication (3-2) presents the first attempts at Genotype-Phenotype correlation between polymorphisms in the GSC and clinical presentation as defined by a variety of clinical subtype groupings. The negative results indicating no significant association between type of mutation, site of mutation, or role in the extracellular or intracellular compartment certainly suggest that the underlying assumptions regarding the role of GSC mutations in HS need further mechanistic scrutiny.

The following three publications (3-3,3-4,3-5) then examine in-silico data

Overall, these publications question the Notch-associated hypothesis of gamma secretase polymorphisms in HS and raises the prospect that GCS substrates other than Notch may be involves in the pathogenesis of HS. This is supported by data showing the low rates of GSC polymorphisms in European cohorts and data demonstrating the PI3K/AKT pathway dysregulation seen in East-Asian cohorts cannot be replicated in tissue samples from over 80 European HS patients[74].

Therefore, the path forward for future work in the genetic basis of HS includes genotype- endotype- phenotype analysis[75], identification of other associated variants targeting proteins other than Notch, GWAS, as well as acknowledgement of the potential genetic heterogeneity in the disease.

4.3.1: Publication 3-1

Frew JW, Vekic DA, Woods J, Cains GD (2017) "A Systematic Review and Critical Evaluation of Reported Pathogenic Sequence Variants in Hidradenitis Suppurativa"

British Journal of Dermatology 2017; DOI:10.1111/bjd.15441

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4.3.2: Publication 3-2

Frew JW, Hawkes JE, Sullivan-Whalen M, Gilleaudeau P, Krueger JG "Inter-Relater Reliability of Phenotypes, and Exploratory Genotype- Phenotype Analysis in Inherited Hidradenitis Suppurativa" Br J Dermatol 2019; 2019 Jan 28. doi: 10.1111/bjd.1769

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4.3.3: Publication 3-3

Frew JW, Navrazhina K, In-Silico Analysis of Gamma-Secretase-Complex Mutations in Hidradenitis Suppurativa Demonstrates Disease-Specific Substrate Recognition and Cleavage Alterations Front Med 2019; DOI: 10.3389/fmed.2019.00206

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BRIEF RESEARCH REPORT
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In silico Analysis of Gamma-Secretase-Complex Mutations in Hidradenitis Suppurativa Demonstrates Disease-Specific Substrate Recognition and Cleavage Alterations

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Background: Familial Hidradenitis Suppurativa and Familial Alzheimer's Disease are both associated with Gamma-Secretase Complex mutations; however, the two diseases are not epidemiologically associated. Understanding the molecular differences between the two diseases may aid in the development of hypotheses for differing pathogenesis and ultimately, targets for detection.

Aims: To characterize the *in silico* structural and functional alterations to the Gamma Secretase Complex in documented mutations in Familial Hidradenitis Suppurativa, along with comparison of downstream substrate recognition and cleavage.

Methods: *In silico* analysis of publicly available genomic data, assessment of protein structure and binding affinity using Swiss-model and Dynamut was undertaken. Differential Expression was expressed using Log Fold Change using the general framework for linear models in R. Differentially expressed genes (DEGs) were defined by FCH $\geq$1.5 or ≤ -1.5 and false discovery rate (FDR $\leq$ 0.05).

Results: Twenty three of 39 mutations in HS are degraded via nonsense mediated decay with altered substrate and binding affinity of substrates identified in the remaining mutations. Significant differential expression of ErbB4, SCNB1, and Tie1 in lesional skin was specific to Hidradenitis Suppurativa and EphB2, EPHB4, KCNE1, LRP6, MUSK, SDC3, Sortilin1 in blood specific to Familial Alzheimer's Disease.

Discussion and Conclusions: We present the first *in silico* evidence as to the impact of documented mutations in Familial Hidradenitis Suppurativa. We also demonstrate unique substrate recognition and cleavage between Hidradenitis Suppurativa and Familial Alzheimer's Disease, providing a potential explanation as to why the two diseases do not occur within the same pedigree. These proteomic signatures may be a first step in identifying reliable biomarkers for Familial Hidradenitis Suppurativa.

Keywords: Hidradenitis Suppurativa, Alzheimer's disease, gamma secretase complex, nicastrin, pre-senilin

INTRODUCTION

Familial Hidradenitis Suppurativa (HS) and Familial Alzheimer's Disease (AlzD) are two inherited diseases associated with mutations in the Gamma Secretase Complex (GSC) (1, 2). The GSC is a transmembrane protease composed of four subunits: presenilin-1 (*PSEN1),* Nicastrin (*NCSTN*), anterior-pharynx-defective 1 (*APH-1*), and presenilin-enhancer-2 (*PEN-2*). Familial HS and AlzD are not epidemiologically associated (3) and no known mutations overlap between the two diseases (4), however the reasons why these two diseases do not co-occur (given that they are both associated with mutations in the GSC) is unknown.

The GSC cleaves up to 69 individual substrates (5), the most well-known being amyloid precursor protein (APP) associated with AlzD (6) pathogenesis. Of interest, altered GSC substrate proteolysis is seen in non-neural tissues (including cutaneous fibroblasts) in AlzD (6) suggesting that peripheral tissues such as cutaneous fibroblasts can be analyzed for diagnostic and predictive biomarkers of disease (6). The structural and functional impacts of GSC mutations in AlzD has been well-characterized through molecular dynamics *in silico* techniques, however, there is a lack of similar studies examining the role of GSC mutations in HS (7, 8).

Limited data exists assessing the impact of mutations on GSC proteolysis in HS epidermal keratinocytes (9), with existing data dependent upon the assumption that Notch signaling (the most studied GSC substrate in HS) is the sole pathogenic mechanism in the disease, which remains unproven (10). There is no known assessment of the impact of HS-associated mutations on other GSC substrates other than Notch (9, 10). Given the documented positive transcriptional feedback mechanisms in proteolyzed GSC substrates such as Notch (11), examining the differential expression of GSC substrates may give an indication as to the specific mechanistic pathways involved in each disorder. The lack of data regarding the effects of HS-associated GSC mutations impairs our ability to understand the molecular pathogenesis of HS, as well as accurately interpret novel peripheral biomarkers specific to HS vs. AlzD. It is also unclear what the normal background variation of GSC substrate expression is in the setting of cutaneous inflammation. This is important in order to interpret the functional significance of differential expression.

AIMS

We aimed to systematically assess all known mutations in the components of the GSC in Familial HS *in silico* for resulting protein structure and binding affinity. We also aimed to compare the downstream GSC substrate recognition and cleavage between HS and AlzD, along with a panel of other most common inflammatory dermatoses (psoriasis, atopic dermatitis, and alopecia areata) and neurodegenerative disorders (Parkinson's disease, Huntington's Disease) for comparison and identification of non-specific background effects.

METHODS

Identification of Sequence Variants in Hidradenitis Suppurativa

Variants identified as pathogenic in our previous systematic review (4) were visually confirmed in the Integrative Genomics Viewer (IGV) version 2.4 (Broad Institute, Cambridge, Massachusetts, USA.). These reviews also assessed the pathogenicity of individual variants using pre-defined consensus criteria of the American College of Medical Genetics and Genomics and Association for Molecular Pathology (11).

FASTA Amino Acid Sequences for Wild Type and Variants

FASTA amino acid sequences for wild type proteins in the GSC were sourced from UniprotKB/Swiss-Prot (www.uniprot.org) with the following Entries: Nicastrin (NCSTN): Q92542; Pre-Senilin 1 (PSEN1): P49768; Pre-Senilin 2(PSENEN): Q9NZ42.

In silico Assessment of Protein Structure and Binding Affinity

Swiss-Model (www.swissmodel.expasy.org) was used in automated mode using FASTA format amino acid sequences to analyze protein conformational change. Those proteins without significant conformational alteration (based on visual inspection) were considered less likely to undergo nonsense mediated decay (NMD) and were then assessed for binding affinity. Dynamut (http://biosig.unimelb.edu.au/dynamut/) was employed using single mutation analysis. Protein Data Bank (PDB) structure of the combined gamma secretase complex was sourced from RCSB PDB (www.rscb.org) with PDB ID.

Identification of Gamma Secretase Complex Substrates

A comprehensive list of GSC substrates was compiled from the existing literature with a total of 69 substrates identified (1).

Gene Expression Data Sources-Skin

Publicly available gene expression data for skin were sourced from NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/) with the following GSE numbers:

- Hidradenitis Suppurativa Lesional Skin- GSE 72702 ($n = 30$).
- Psoriasis Lesional Skin- Krueger GSE 13355 ($n = 122$).
- Alopecia Areata Lesional Skin: GSE 45512 ($n = 10$).
- Atopic Dermatitis Lesional Skin GSE 32924 ($n = 22$).

Normal Unaffected Controls were pooled from GSE 13355, 45512, 32924 to use as a common reference. Non lesional samples (including GSE 72702) were excluded from analysis. General Hidradenitis Suppurativa gene expression data was used as no specific gene expression data is available for Familial HS patients.

Gene Expression Data Sources-Blood

Publicly available gene expression data for whole blood were sourced from NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/) with the following GSE numbers and references:

- Hidradenitis Suppurativa Whole Blood: GSE 79149 ($n = 26$).
- Alzheimer's Disease Whole Blood: Mukhamedyarov et al. (12) ($n = 10$).
- Parkinson's Disease Whole Blood GSE 54536 ($n = 10$).
- Huntington's Disease Whole Blood: GSE 24250 ($n = 14$).

Normal Unaffected Controls were pooled from GSE 79149, GSE 54536, and GSE 24250 and reference 2 above to use as a common normal reference.

Statistical Analyses

Statistical Analysis was performed using the standard R package (R Core Team, 2019). Differential gene expression between normal unaffected controls and HS, inflammatory dermatoses and neurodegenerative disorders was performed. Visualization of data was performed with GraphPad Prism 7 software (GraphPad Software). Expression values were modeled using a mixed-effects model with lesional categories as fixed factors and random effects for each patient. Fold Changes (FC) were estimated under the general framework for linear models in the R limma package with heteroscedasticity accounted for using parameter ArrayWeights. Batch effect was assessed and removed using the R limma package removeBatchEffect function. *P*-values from *t*-tests were adjusted for multiple hypotheses using Benjamini–Hochberg procedure. Differentially expressed genes (DEGs) were defined by FC $\geq$ 1.5 or $\leq$-1.5 and false discovery rate (FDR $\leq$ 0.05). Statistical comparison of substrate expression between conditions was conducted using one way ANOVA with $p < 0.05$ considered significant.

Further pathway analysis and assessment of upstream regulators was performed using the Ingenuity Pathway Analysis (IPA) Tool (Ingenuity H Systems, Redwood City, CA). Differentially expressed GSC substrates in HS and AlzD were analyzed to identify activated or suppressed biological pathways using IPA algorithms. Predicted activation scores (z scores >2 or <2) were considered significant and the description of pathways are based upon IPA algorithms and output.

RESULTS

The normal structure of the GSC is presented in **Figures 1A,B**. Representative alterations found in HS in Nicastrin (**Figures 1C–E**), Pre-senilin 1 (**Figures 1F,G**), and Pre-senilin 2 (**Figures 1H–J**) demonstrating particular mutations with either minimal or significant structural alterations are shown. The complete list of structural alterations are presented in **Supplementary Figures 1**, **2**. Structural analysis suggested 21 of 30 *NCSTN*, 0 of 3 *PSEN1* and 2 of 6 *PEN-2* mutations in HS are degraded via NMD (**Supplementary Figures 1**, **2**).

Representative results of binding and substrate affinity alterations in HS in the 16 variants not degraded by NMD are presented in **Figures 1K–N**. The complete list is available in **Supplementary Figure 3**. Binding and flexibility alterations to the trans-membrane domain (TMD) of Nicastrin, Pre-senilin1, and Pre-senilin-2 were identified (**Figures 1K–N**), as well as binding alterations to potential substrate binding sites in the extracellular domains of the respective proteins (E3 for Pre-senilin 1 NTF, H6 for Nicastrin, A30 for Pre-senilin 2) (7, 14).

Differentially expressed GSC substrates specific to HS and AlzD are presented in **Figures 2A,B**, and the complete heatmap of GSC substrate differential expression is presented in **Figure 2C**. Significant differential expression of ErbB4, SCNB1, and Tie1 in lesional skin was specific to HS and EphB2, EPHB4, KCNE1, LRP6, MUSK, SDC3, Sortilin1 in blood specific to AlzD. Other inflammatory dermatoses (psoriasis, alopecia areata, and atopic dermatitis) as well as neurodegenerative disorders (Parkinson's disease, Huntington's disease) included for comparison of non-specific background effects. Significant differential expression between HS and inflammatory dermatoses ($P < 0.001$), as well as AlzD and other neurodegenerative disorders ($p < 0.05$) was significant by one-way ANOVA.

In order to account for the possibility of differential cleavage of substrates altering the function (but not the total amount) of GSC substrates, we compared activated and suppressed pathways downstream of GSC substrate cleavage between AlzD and HS. Activated pathways associated with HS included apoptosis, "apoptosis of fibroblast cell lines," "cell death of connective tissues," "cell proliferation of fibroblasts," and "binding of immune cells" as per Ingenuity Pathway Analysis. These were associated with the ErBB4, IFNAR1 IFNAR2, IL1R1 IL1R2, IGF1R substrates (The complete list of associated substrates associated with these pathways are available in **Supplementary File 2**). These pathways have previously been implicated in NCSTN knockdown cell lines, independently validating the results of our *in silico* methods (15). Activated pathways in AlzD included invasion of carcinoma cells, proliferation of connective tissues, invasion of tumor cells, and movement disorders (**Supplementary File 2**). These were associated with APP, CD44, AXL, CSF1R, MET, TGFBR3 substrates (**Supplementary File 2**). Common pathways which were differentially activated or suppressed in blood between AlzD and HS as inidicated in Ingenuity Pathway Analysis included: "advanced malignant tumor," "cellular infiltration by leucocytes," "metastasis of cell lines," "movement disorders," and "secondary tumors" (**Supplementary Figure 4**). (The complete list of substrates associated with these pathways are available in **Supplementary File 2**).

DISCUSSION

In silico analysis of HS-associated mutations in GSC identifies significant structural and functional alterations consistent with known sites of substrate binding and cleavage. Even in the setting of NMD of one component of the GSC, membrane localization of GSC is known to occur, albeit with altered proteolytic activity (14). NMD of NCSTN (as one of the most common results of HS associated mutations) is anticipated to increased substrate cleavage through the removal of the NCSTN extracellular domain "gatekeeper" (14) but may also reduce cleavage through the removal of extracellular substrate binding sites.

Unique HS-associated differential expression of GSC substrates was identified for ErbB4, SCN1B, and Tie1, although significant differential expression of multiple other substrates were seen in other inflammatory dermatoses. Given the lack of (known) altered GSC complex activity in these inflammatory

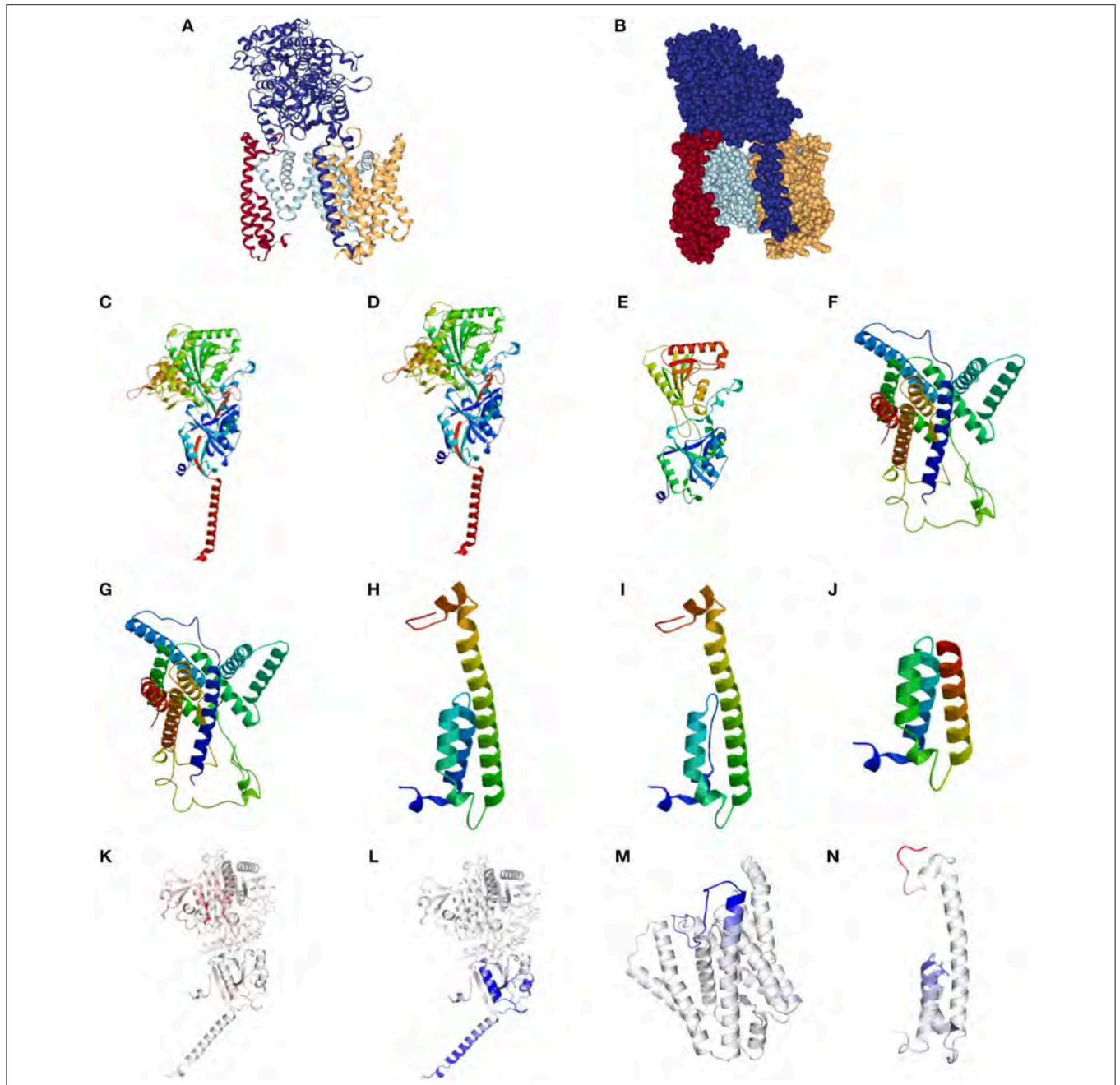

FIGURE 1 | Structural and binding stability assessments of identified mutations in Familial HS. **(A)** Presents the structure of the gamma secretase complex (GSC) with Nicastrin (Dark Blue), PSEN1 (Light Blue), PSENEN (Red), and APH1 (Yellow). The "V" shaped transmembrane domain cleave site can be identified in light blue. Filled structure **(B)** demonstrates the binding pocket with access to the PSEN1 substrate cleavage site, surrounded by PSNEN and *NCSTN* substrate binding sites. Wild Type (WT) *NCSTN* **(C)**, *NCSTN* V75I **(D)**, and *NCSTN* Q420X **(E)**, PSEN1 WT **(F)**, *PSEN1* 953A>G **(G)**, *PSENEN* WT **(H)**, *PSENEN* 43_56del14 **(I)**, *PSENEN* 66delG **(J)**. Binding and affinity assessment of *NCSTN* 996+7G>A **(K)**, *NCSTN* V75I **(L)**, *PSEN1* 725delC **(M)**, *PSENEN* 66_67insG **(N)**. Blue indicates decreased binding affinity and increased flexibility with red indicating increased binding affinity with decreased flexibility. For comprehensive conformational alterations in AlzD the reader is referred to Berezovska et al. (13).

disorders, this may indicate possible non-specific effects of inflammation upon expression and function of GSC substrates which warrants further investigation. This also brings into question whether alterations in substrates such as Notch are specific to disease (10), or rather a non-specific inflammation related finding. Similar non-specific background effects were also seen in neurodegenerative disorders such as Huntington's and Parkinson's Disease.

The identification of HS-specific substrates also raises the possibility that some of these substrates which are druggable

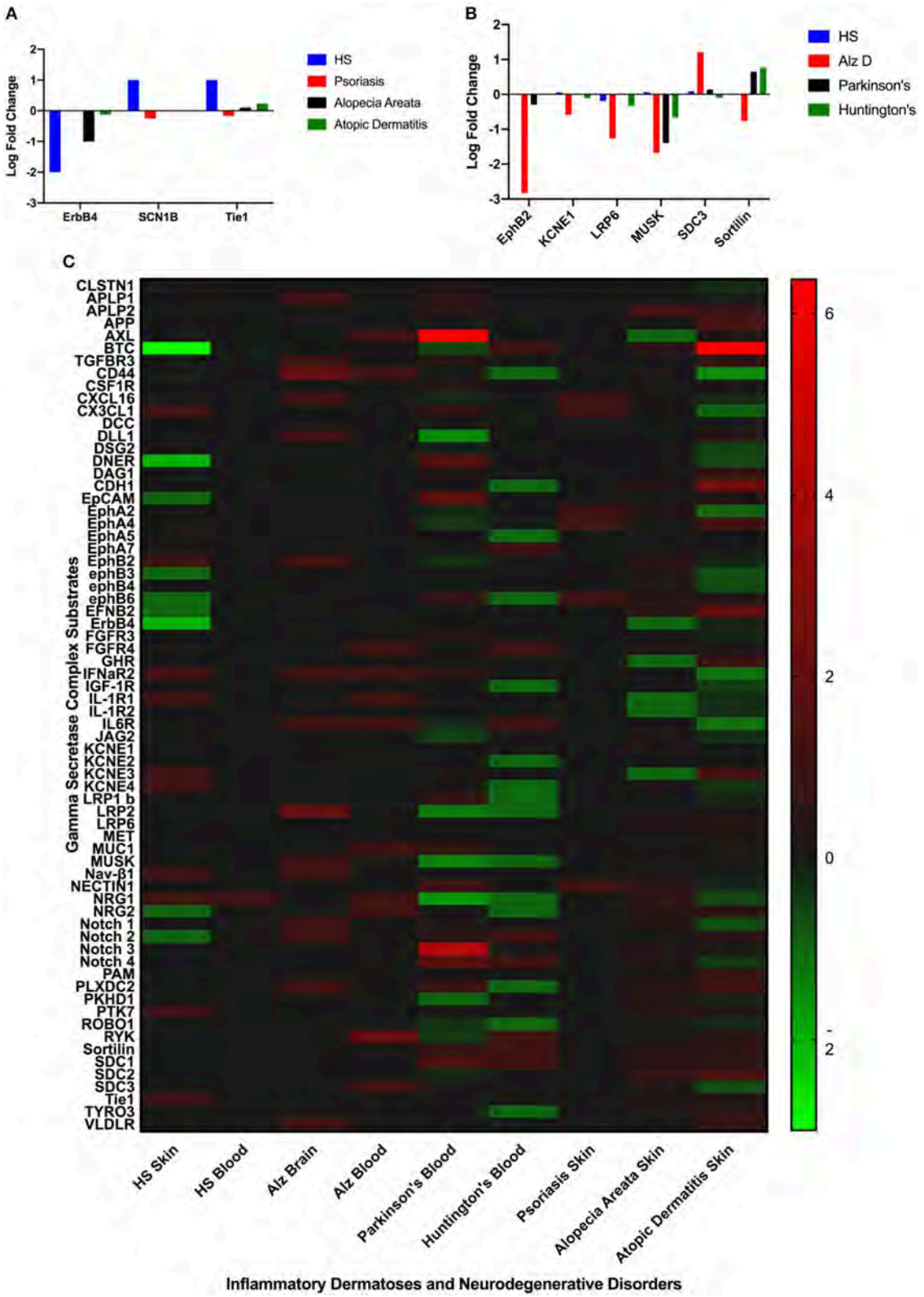

FIGURE 2 | Comparison of significantly differentially expressed GSC substrates across inflammatory skin disease **(A)**, and neurodegenerative disorders **(B)**. Only the substrates specific to HS **(A)** or AlzD **(B)** are illustrated. Significant differential expression of ErbB4, SCNB1, and Tie1 in lesional skin was specific to Hidradenitis Suppurativa and EphB2, EPHB4, KCNE1, LRP6, MUSK, SDC3, Sortilin1 in blood specific to Familial Alzheimer's Disease. A Heatmap **(C)** of all GSC substrates across all measured datasets highlighting the non-specific differential expression in a number of disorders not associated with inherited mutations in the GSC.

targets (such as IGF-1R, Tie1) may represent novel therapeutic approaches. CSF1R, IFNAR1 IFNAR2, IL1R1 IL1R2 pathway upregulation is greater in HS than AlzD, consistent with its role in systemic inflammation. Interferon responsive pathways have been independently documented in NCSTN shRNA knockdown keratinocyte cell lines (16) supporting the role of these pathways in HS. Diabetes and obesity are also associated with HS (17), implicating IGF1R. Follicular hyperkeratinization, prominent dermal fibrosis and dermal tunnel formation in HS have led to hypotheses of Wnt signaling deficiencies in dermal fibroblasts and mesenchymal cells of the dermal papillae (18). FGFR4, MUC1, and MUSK pathway downregulation provide evidence to support this hypothesis. These results suggest that despite the inherent limitations of an *in silico* analysis of existing genomic data, this approach is capable of identifying key targets in disease.

The clinical validity of this work is supported by the fact that all assessed sequence variants are those deemed pathogenic through established criteria as published in our previous review (11), although a limitation to our study is that no external validation of functional confirmation has been undertaken to confirm these *in silico* findings. We note with interest that Notch was not identified as a HS-specific GSC substrate, despite the evidence from the published literature (19, 20) regarding alterations in Notch signaling and POGLUT1 (20), an endoplasmic reticulum O-glucosyltransferase involved in Notch signaling. This may indicate that Notch is not a HS- specific substrate and alterations in Notch and subsequent Notch-associated loci (21) may also be shared with AlzD. Further investigation into the role of Notch signaling across inflammatory dermatoses and neurodegenerative disorders may be informative in this regard.

CONCLUSION

The results of our *in silico* analysis identify that HS-associated mutations have structural and potentially functional impact upon GSC substrates. These effects are distinct between HS and AlzD which explains their lack of co-occurrence in pedigrees. Our data identifies the differential expression of specific substrates, which may function as proteomic signatures of disease. Further prospective studies are needed to validate these targets. The downstream affected pathways confirm the previous experimental results of NCSTN knockdown cell lines (15) giving validation to our approach. Our results present a first step in understanding the molecular pathogenesis of Familial HS with a view toward diagnostic biomarkers of disease.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here (**Supplementary File 1**): Publicly available gene expression data for skin were sourced from NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/) with the following GSE numbers:

- Hidradenitis Suppurativa Lesional Skin- GSE 72702.
- Psoriasis Lesional Skin GSE 13355.
- Alopecia Areata Lesional Skin: GSE 45512.
- Atopic Dermatitis Lesional Skin GSE 32924.
- Hidradenitis Suppurativa Whole Blood: GSE 79149.
- Alzheimer's Disease Whole Blood: Mukhamedyarov et al. (12).
- Parkinson's Disease Whole Blood GSE 54536.
- Huntington's Disease Whole Blood: GSE 24250.

AUTHOR CONTRIBUTIONS

JF and KN designed the study. JF performed the analysis and wrote the manuscript. KN made revisions to the manuscript. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00206/full#supplementary-material

Supplementary Figure 1 | Nicastrin morphological alterations by SWISS MODEL.

Supplementary Figure 2 | PSEN1 and PSENEN morphological alterations by SWISS MODEL.

Supplementary Figure 3 | Binding and flexibility assessment by dynamut for all proteins not undergoing NMD.

Supplementary Figure 4 | Comparison of functional annotation for HS and Alzheimer's disease.

Supplementary File 1 | Raw data of FCH and LogFCH in Gamma Secretase Substrates across HS, AlzD, Psoriasis, AD, Alopecia Areata, Parkinson's disease, and Huntington's disease.

Supplementary File 2 | ADAM10 and ADAM17 expression between HS, AzD, Psoriasis, Atopic Dermatitis, Alopecia Areata, Parkinson's disease, and Huntington's disease.

REFERENCES

1. Wang B, Yang W, Wen W, Sun J, Su B, Liu B, et al. Gamma-secretase gene mutations in familial acne inversa. *Science*. (2010) 330:1065. doi: 10.1126/science.1196284

2. Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, et al. Early-onset autosomal dominant Alzheimer's disease: prevalence, genetic heterogeneity and mutation spectrum. *Am J Hum Genet*. (1999) 65:664–70. doi: 10.1086/302553

3. Theut Riis P, Egeberg A, Gislason GH, Jemec GB. Patients with hidradenitis supurativa have no increased risk of Alzheimer's disease. *Br J Dermatol*. (2017) 273–5. doi: 10.1111/bjd.15064
4. Frew JW, Vekic DA, Woods JA, Cains GD. A systematic review and critical evaluation of reported pathogenic sequence variants in Hidradenitis Suppurativa. *Br J Dermatol*. (2017) 177:987–98. doi: 10.1111/bjd.15441
5. Merilahti JAM, Ojala VK, Kittle AM, Pulliainen AT. Genome wide screen of gamma secretase mediated intramembrane cleavage of receptor tyrosine kinases. *Mol Biol Cell*. (2017) 28:3123–31. doi: 10.1091/mbc.e17-04-0261
6. Mocali A, Malva ND. Altered proteolysis in fibroblasts of Alzheimer patients with predictive implications for subjects at risk of disease. *Int J Alzheimer's Dis*. (2014) 2014:520152. doi: 10.1155/2014/520152
7. Inoue K, Oliviera LMA, Abeliovich A. CRISPR transcriptional activation analysis unmasks an occult gama-secretase processivity defect in familial Alzheimer's disease skin fibroblasts. *Cell Rep*. (2017) 21:1727–36. doi: 10.1016/j.celrep.2017.10.075
8. Fukumori A Steiner H. Substrate recruitment of y-secretase and mechanisms of clinical presenilin mutations revealed by photoaffinity mapping. *EMBO J*. (2016) 35:1628–43. doi: 10.15252/embj.201694151
9. Xiao X, He Y, LI C, Zhang X, Xu H, Wang B. Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide-3-kinase/AKT signaling pathways. *Br J Dermatol*. (2016) 174:522–32. doi: 10.1111/bjd.14223
10. Melnik BC, Plewig G. Impaired Notch signaling: the unifying mechanisms explaining the pathogenesis of Hidradenitis Suppurativa. *Br J Dermatol*. (2013) 168:876–8. doi: 10.1111/bjd.12068
11. Chen KY, Srinivasan T, Tung KL, Belmonte JM, Wang L, Murthy PKL, et al. A Notch positive feedback in the intestinal stem cell niche is essential for stem cell self-renewal. *Mol Syst Biol*. (2017) 13:927 doi: 10.15252/msb.20167324
12. Mukhamedyarov MA, Rizvanov AA, Yakupov EZ, Zefirov AL, Kiyasov AP, Reis HJ, et al. Transcriptional analysis of blood lymphocytes and skin fibroblasts, keratinocytes and endothelial cells as potential biomarkers for Alzheimer's disease. *J Alzheimers Dis*. (2016) 54:1373–83. doi: 10.3233/JAD-160457
13. Berezovska O, Lleo A Herl LD Frosch MP, Stern EA, Bacskai BJ, et al. Familial Alzheimer's disease presenilin 1 mutations cause alterations in the conformation of presenilin and interations with amyloid precursor protein. *J Neurosci*. (2005) 25:3009–17. doi: 10.1523/JNEUROSCI.0364-05.2005
14. Shah S, Lee SF, Tabuch K, Hao YH, Yu C, LaPlant Q, et al. Nicastrin functions as a gamma secretase substrate receptor. *Cell*. (2005) 122:435–47. doi: 10.1016/j.cell.2005.05.022
15. Cao L, Morales-Heil DJ, Roberson EDO. Nicastrin haploinsufficieny alters expression of type 1 interferon-stimulated genes: the relationship to familial Hidradenitis Suppurativa. *Clin Exp Dermatol*. (2019) 44:e118–25. doi: 10.1111/ced.13906
16. Kanni T, Zenker O, Habel M, Riedemann N, Giamarellos-Bourboulis EJ. Complement activation in Hidradenitis Suppurativa: a new pathway of pathogenesis? *Br J Dermatol*. (2018) 179:413–9. doi: 10.1111/bjd.16428
17. Saunte DML, Jemec GBE. Hidradenitis Suppurativa: advances in diagnosis and treatment. *JAMA*. (2017) 318:2019–32. doi: 10.1001/jama.2017.16691
18. Frew JW, Navrazhina K, Marohn M, Lu PC, Krueger JG. Contribution of fibroblasts to tunnel formation and inflammation in Hidradenitis Suppurativa/acne inversa. *Exp Dermatol*. (2019) 28:886–91. doi: 10.1111/exd.13978
19. Jfri A, O'Brien E, Litvinov I, Alavi A, Netchiporouk E. Hidradenitis Suppurativa: comprehensive review of predisposing genetic mutations and changes. *J Cut Med Surg*. (2019) 23:519–27. doi: 10.1177/12034754198 52049
20. Li X, Jiang L, Huang Y, Ren Z, Liang X, Wang P. A gene dysfunction module reveals the underlying pathogenesis of Hidradenitis Suppurativa: an update. *Australas J Dermatol*. (2019). doi: 10.1111/ajd.13107. [Epub ahead of print].
21. Gauntner TD. Hormonal, stem cell and Notch signaling as possible mechanisms of disease in Hidradenitis Suppurativa: a systems level transcriptomic analysis. *Br J Dermatol*. (2019) 180:203–4. doi: 10.1111/bjd.17093

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.3.4: Publication 3-4

Frew JW "We Need to Talk About Notch: Notch Dysregulation as an Epiphenomenon in Inflammatory Skin Disease" (2018) Br J Dermatol 2019 Feb;180(2):431-432

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Article Available on Publisher Website:
https://onlinelibrary.wiley.com/doi/10.1111/bjd.17414

4.3.5: Publication 3-5

Frew JW Navrazhina K No Evidence that Impaired Notch Signaling Differentiates Hidradenitis Suppurativa from other Inflammatory Skin Diseases Br J Dermatol 2020;182(4):1042-1043

Article Not Included due to Copyright Restrictions.
Article Available on Publisher Website:
https://onlinelibrary.wiley.com/doi/10.1111/bjd.18593

4.4: Clinical Factors Influencing Clinical Response to Therapy in HS:

Manuscript	Manuscript Reference
4-1	Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger JG Dermal Tunnels Influence Time to Clinical Response and Family History Influences Time to Loss of Clinical Response in Hidradenitis Suppurativa Patients Treated with Adalimumab. Clin Exp Dermatol 2020; 46(2):306-313
4-2	Frew JW, Singh N, Jiang CS, Navrazhina K, Vaughan R, Krueger JG. The Impact of Body Mass Index Upon the Efficacy of Adalimumab in Hidradenitis Suppurativa. Front Medicine 2021;8:603281
4-3	Frew JW, Jiang C, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger J "Malignancy and Infection Risk During Adalimumab Therapy in Hidradenitis Suppurativa" Clin Exp Dermatol 2020; 45(7):859-865

Table 4-4-1 Publications Presented in this Chapter

The efficacy of existing therapies in HS is low compared to other inflammatory disease such as Psoriasis Vulgaris and Atopic Dermatitis[76]. Hence the identification of potential clinical characteristics of biomarkers which may be associated with positive or negative treatment response have potential benefits to the overall management strategies in the care of HS patients. Currently the therapeutic guidelines for HS suggest multimodal approaches involving lifestyle, anti-inflammatory, anti-microbial and surgical modalities[27-31]. This reflects our poor understanding of the pathogenesis of the disease as well as the relatively low level of therapeutic efficacy of the currently available therapeutic agents.

The publications comprising this chapter utilise de-identified patient data from the pivotal Phase 3 clinical trials of Adalimumab in Hidradenitis Suppurativa to examine three major aspects of clinical safety and efficacy.

The first publication (4-1) uses a survival analysis methodology to re-examine the factors contributing to time to clinical response (as defined by both the HiSCR and IHS4) and time to loss of clinical response. This was to address a significant gap in the existing published data where the use of last observation carried forward (LOCF) methods may have overinflated the clinical response rates in the open-label extension study of Adalimumab in HS. The published results were certainly in contrast to what was observed in clinical practice, where Adalimumab demonstrated a waning degree of efficacy over time. The results from this study indicate that dermal tunnels was a significant variable in time to achieving clinical response to Adalimumab and family history was a significant variable in accelerating the time to loss of clinical response. These findings were consistent with what is largely observed that individual patients with more severe, extensive disease and those with a strong family history are more recalcitrant to therapy. This is reflected in the results of paper 4-1 where the majority of patients exited the trial due to lack of therapy.

The second publication (4-2) critically evaluated the influence of Body Mass Index (BMI) in the efficacy of Adalimumab in HS. Previous discussion has focused around whether individuals with greater BMI require higher dosing of existing therapies in HS. The results from this re-analysis were complex, but largely indicate that BMI is a significant covariate in achieving clinical response to Adalimumab. However, it is largely a product of the outcome measure itself, and only significant at lower baseline disease activity.

There was no evidence of significant differential cut-offs for BMI in ROC analysis which would indicate a specific BMI or weight at which higher doses of Adalimumab may be beneficial.

The third publication (4-3) objectively examined a common safety aspect of Adalimumab therapy, being malignancy and infection risk. A common concern from patients and physicians alike is the increase in infection and malignancy risk in the setting of Adalimumab therapy. By examining the disease-specific and treatment-specific incidence rates of malignancy and infection and comparing them to other inflammatory diseases (such as rheumatoid arthritis, Crohn's disease) there is evidence that the elevation in malignancy and infection risk is a feature of Hidradenitis Suppurativa more so than therapy with Adalimumab. This has direct implications for the counselling of patients prior to Adalimumab prescribing as well as the understanding of malignancy and infection risk in untreated disease.

4.4.1: Publication 4-1

Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger JG Dermal Tunnels Influence Time to Clinical Response and Family History Influences Time to Loss of Clinical Response in Hidradenitis Suppurativa Patients Treated with Adalimumab. Clin Exp Dermatol 2020; 46(2):306-313

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https://onlinelibrary.wiley.com/doi/10.1111/ced.14448

4.4.2: Publication 4-2

Frew JW, Singh N, Jiang CS, Navrazhina K, Vaughan R, Krueger JG. The Impact of Body Mass Index Upon the Efficacy of Adalimumab in Hidradenitis Suppurativa. Front Medicine 2021; 8:603281

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 22 June 2021
doi: 10.3389/fmed.2021.603281

The Impact of Body Mass Index Upon the Efficacy of Adalimumab in Hidradenitis Suppurativa

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Elevated BMI in Hidradenitis Suppurativa is associated with decreased response to Adalimumab therapy. BMI is proposed to segregate distinct disease subtypes. It remains unresolved whether a threshold BMI exists above which increased dosages may provide clinical benefit. Individual patient data from 578 PIONEER Phase 3 participants were analyzed. Descriptive, multivariable regression analysis and receiver operating characteristic (ROC) curves were calculated to assess the relationship between BMI and clinical outcome measures using R v3.5.3. Participants in the overweight and obese BMI category had reduced odds (58 and 67%, respectively) of achieving HiSCR [OR = 0.42 (95%CI −0.19, 0.91) $p = 0.03$], [OR = 0.33 (95%CI 0.16, 0.67) $p = 0.002$] compared to participants with BMI < 25. Reduction in AN count and IHS4 score was not significantly associated. ROC analysis did not reveal any cut off value predictive of treatment outcome. No correlation between BMI and baseline disease activity or covariate interactions were identified. These findings suggest BMI is a significant covariate in the setting of lower baseline disease activity, supporting the concept of disease heterogeneity and differential therapeutic response to Adalimumab.

Keywords: hidradenitis suppurativa, acne inversa, adalimumab, body mass index, pharmacoepidemiogy

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INTRODUCTION

Hidradenitis Suppurativa (HS) is a chronic inflammatory disorder associated with obesity (1, 2) with increasing Body Mass Index (BMI) associated with increasing disease severity (3). Treatment guidelines for moderate to severe disease include the use of TNF-alpha antagonists (4) (Adalimumab) dosed at 40 mg every week in line with the pivotal PIONEER phase 3 clinical studies (5). Infliximab, also a TNF-alpha antagonist is used at a weight based dosage (6) (5 mg/kg/dose) in multiple inflammatory disorders, including psoriasis, rheumatoid arthritis and Crohn's disease, however increased disease control in HS is demonstrated at higher and more frequent dosages (up to 10 mg/kg every 6 weeks) (7). In Phase 2 studies the highest dosage administered was 40 mg per week and hence no data is available on the relative efficacy of doses >40 mg per week (8). Given that both Adalimumab and Infliximab are both TNF-alpha inhibitors, it is not unreasonable to assume that better disease control may be seen with increased dosages of Adalimumab particularly in patients with greater BMI. Additionally, our recent investigations have identified BMI as significantly associated with achieving clinical outcomes as measured by Hidradenitis Suppurativa Clinical Response (HiSCR), which is currently the gold-standard outcome for HS clinical trials (9). Other assessed clinical outcomes include change in abscess

and nodule (AN) count, and the international hidradenitis suppurativa severity score (IHS4). As assessed using logistic regression, every unit increase in BMI significantly reduces the odds of achieving HiSCR by 7.1%. (OR = 0.93; 95% CI: 0.89, 0.97; $p < 0.001$). However, it is unclear whether this relationship is linear (as assumed above) or demonstrates increased significance above a certain threshold. Such relationships are assumed to be linear when analyzed by continuous variables, but their true relationship is only ascertained through more comprehensive analysis. It has also been reported that BMI may segregate distinct HS subtypes (10). Disease characteristics and comorbidities vary between HS patients with low (<30 mg/kg) and high (>35 mg/kg) BMI in case-control studies (10). Data supporting differential disease characteristics and response to therapy may support the premise that pathogenic heterogeneity exists, driving the search for more targeted individualized therapies in this often-recalcitrant disease.

Overall, the characteristics of the relationship between BMI and clinical response to Adalimumab in HS requires further detailed examination. Such statistical evaluation will enable us to accurately assess whether evidence exists for increased dosages of adalimumab beyond 40 mg per week, and for which patient populations this may be beneficial. Our overall aim is to characterize the relationships between BMI and clinical response (as measured by the HiSCR and IHS4) and investigate if a specific BMI cut-off can be identified which is associated with a reduction in clinical response. This would identify a patient subpopulation which may benefit from investigations into an increased dose of Adalimumab therapy.

MATERIALS AND METHODS

De-identified individual patient data (IPD) from the PIONEER 1 and PIONEER 2 Phase 3 studies of Adalimumab therapy in HS (5) were made available by AbbVie Inc. and accessed through the secure Vivli online platform. Raw data were extracted and compared to the available published data (5) to ensure accuracy. Only data for "Time Period A" (Week 0–Week 12) comparing Adalimumab 40 mg weekly vs. placebo was included in the analysis in order to reflect approved dosing regimens. Individuals with incomplete data and those who received antibiotic therapy in PIONEER 1 and those administered every-other-week (EOW) dosing were excluded from analysis. Antibiotic therapy in PIONEER 2 was included as a covariate. BMI was calculated as a continuous variable as well as a categorical variable in line with standardized BMI Categories (<25.0 kg/m^2; 25.0 to <30.0 kg/m^2; ≥30.0 kg/m^2) (11) consistent with CDC and WHO recommendations. Due to the small number of subjects with underweight BMI (<18.5 kg/m^2), this underweight category was merged with the normal BMI (18.5 to <25.0 kg/m^2). All data analysis was conducted in R version 3.5.3 (12).

Each variable of interest was assessed for normality using the Shapiro-Wilk test and histograms. The differences between treatment groups were compared using Student's *t*-test for normally distributed continuous variables and the Mann-Whitney *U*-test for non-normally distributed continuous variables. Chi-squared and Fisher's exact-tests were used for categorical variables. Potential association of body mass index [BMI] variable with HiSCR response and IHS4 response, were assessed using Student's *t*-test for continuous BMI variable and Chi-squared-test for categorical BMI variables. Association of categorical BMI with absolute change in nodule counts and percentage change in IHS4 were assessed using one-way analysis of variance test (ANOVA) and *post-hoc* multiple comparisons tests were performed using Dunnett's method with underweight/normal BMI category as the reference group. Potential associations with categorical BMI, as well as other a priori potential associations [age, sex, Hurley stage, smoking status, family history, antibiotic use (PIONEER 2 only), and presence of draining tunnels] were assessed using logistic regression for HiSCR and binary IHS4 and linear regression for percentage change in IHS4 and absolute change in nodule count. Receiver operating characteristic (ROC) curve analysis was used for examining the best cutoff of BMI for predicting HiSCR and IHS4 response. Likelihood ratio tests and multiple partial F tests were performed to assess whether categorical BMI had a significant impact on the outcome of disease activity when adjusting for a priori covariates. The association of categorical BMI with absolute AN (abscess and nodule) count and IHS4 score was assessed using the Kruskal-Wallis H test. Spearman's rank order correlation analysis was performed to assess the correlation between continuous BMI and absolute disease activity variables. $P < 0.05$ was considered statistically significant.

RESULTS

For the purposes of our analysis, 144 and 145 individuals were included in the Adalimumab and placebo arms, respectively, of PIONEER 1; with 149 and 140 individuals included in the Adalimumab and Placebo arms, respectively, of PIONEER 2. The demographic and disease characteristics of these populations are included in **Table 1**. There was no statistically significant difference between the Adalimumab and placebo arms with regards to demographic and disease characteristics in both PIONEER 1 and PIONEER 2 (**Table 1**), although as previously reported (9) significant differences in race, median age, median BMI, nicotine use, median nodules and draining tunnels exist between PIONEER 1 and PIONEER 2 cohorts (9) (**Supplementary Table 2**).

No statistically significant association was identified between BMI (both as a continuous and a categorical variable) and participants achieving HiSCR in PIONEER 1 (**Table 2**). Similarly, no significant association was seen between BMI (both as a continuous and a categorical variable) and patients achieving IHS4 category change in PIONEER 1 (**Table 2**). A statistically significant association was seen between BMI as a continuous variable and achieving HiSCR (29.9 vs. 32.6, $p < 0.001$) and achieving IHS4 category change (30.0 vs. 32.2 $p = 0.004$) in PIONEER 2. This significant association also held when BMI was analyzed as a categorical variable against achieving HiSCR ($p = 0.01$) and IHS4 category change ($p = 0.03$; **Table 2**). The overall

TABLE 1 | Characteristics of population in each of the trial data.

Characteristic	PIONEER 1			PIONEER 2		
	Adalimumab	**Placebo**	***P*-value**	**Adalimumab**	**Placebo**	***P*-value**
N =	144	145		149	140	
Female	85 (59.0%)	100 (69.0%)	0.10	97 (65.1%)	98 (70.0%)	0.45
Male	59 (41.0%)	45 (31.0%)		52 (34.9%)	42 (30.0%)	
White	111 (77.1%)	113 (77.9%)	0.35	130 (87.2%)	110 (78.6%)	0.07
Black	30 (20.8%)	25 (17.2%)		8 (5.4%)	18 (12.9%)	
Other	3 (2.1%)	7 (4.8%)		11 (7.4%)	12 (8.6%)	
Median age	35.0 (28.0, 45.0)	37.0 (30.0, 47.0)	0.14	35.0 (27.0, 42.0)	35.0 (26.0, 43.25)	0.49
Median BMI	32.1 (27.1, 38.0)	33.9 (28.5, 39.4)	0.07	30.3 (26.3, 36.0)	31.3 (26.8, 36.0)	0.22
BMI category						
Underweight/normal	24 (16.7%)	12 (8.3%)	0.09	32 (21.5%)	25 (17.9%)	0.43
Overweight	30 (20.8%)	36 (24.8%)		40 (26.8%)	32 (22.9%)	
Obese	90 (62.5%)	97 (66.9%)		77 (51.7%)	83 (59.3%)	
Hurley 2	80 (55.6%)	79 (54.5%)	0.95	76 (51.0%)	79 (56.4%)	0.42
Hurley 3	64 (44.4%)	66 (45.5%)		73 (49.0%)	61 (43.6%)	
Nicotine Use	77 (53.5%)	88 (60.7%)	0.26	96 (64.4%)	99 (70.7%)	0.31
Family History	37 (25.7%)	28 (19.3%)	0.25	36 (24.2%)	39 (27.9%)	0.56
Presence of Draining Tunnels	108 (75.0%)	108 (74.5%)	1.00	99 (66.4%)	87 (62.1%)	0.52
Antibiotics	–	–		27 (18.1%)	28 (20.0%)	0.80
Median nodules	8 (4.75, 14)	7 (4, 15)	0.88	6 (4, 11)	6 (4, 10.25)	0.98
Median abscesses	1.5 (0, 4)	2 (0, 3)	0.77	1 (0,3)	1 (0,3)	0.88
Median draining tunnels	2.5 (0.75, 7)	2 (0, 5)	0.38	2 (0, 4)	1 (0, 4)	0.60
Median baseline IHS4	26.5 (15, 45.25)	25 (12, 40)	0.28	19 (10, 34)	18 (8.75, 32.25)	0.91

trend was for less patients achieving HiSCR or IHS4 category change with increasing BMI.

No significant association were identified between BMI categories and % change in AN count or % change in IHS4 count in PIONEER 1, but both comparisons were significant in PIONEER 2 (**Table 2**). *Post-hoc* Dunnett's-test between BMI categories and % change in AN count (obese vs. underweight/normal, $p = 0.01$) or % change in IHS4 count in Pioneer 2 (obese vs. underweight/normal, $p = 0.03$) showed significant difference between obese BMI category and underweight/normal BMI category. There was no difference between overweight vs. underweight/normal in *post-hoc* testing for the % change in AN count and % change in IHS4 count. Both the % change in AN count and % change in IHS4 count reduced with increasing BMI in PIONEER 2 whereas this trend was not seen in PIONEER 1 data (**Table 2**).

Logistic and Linear Regression Analysis examining BMI as categorical variables identified participants in the overweight and obese BMI categories as associated with decreased odds of achieving HiSCR compared to participants with BMI < 25 (**Table 3**). Participants in the overweight BMI Category had a reduction in the odds of achieving HiSCR of 58% [OR = 0.42 (95%CI 0.19, 0.91) $p = 0.03$] and participants in the obese category had a reduction in the odds of achieving HiSCR by 67% [OR = 0.33 (95%CI 0.16, 0.67) $p = 0.002$] compared to participants with BMI < 25 (**Table 3**). Categorical BMI demonstrated an association with achieving IHS4 category change; participants in the overweight BMI category had an increase in the odds of achieving IHS4 category change of 113% [OR = 2.13 (95% CI 1.16, 3.95) $p = 0.02$] as compared to obese BMI category. Overweight BMI was also significant as assessed using the likelihood ratio test.

In analysis with both categorical and continuous BMI there was no significant association with change in AN count (**Table 4**). However, in percentage change in IHS4 score, each unit increase in BMI (as a continuous variable) attenuated the percentage reduction in IHS4 score by 1.65% ($b = 1.65$; 95% CI: 0.50, 2.81; $p = 0.01$). The multiple partial *F*-test suggested that categorical BMI did not have a significant effect on the percentage change in IHS4 when adjusting for covariates.

Receiver Operating Characteristic Curve Analysis

Given the discrepancies between continuous and categorical BMI, we enquired as to whether a specific BMI could be identified as the point with the most appropriate cut off for specific measurements of disease activity (HiSCR and IHS4). Through ROC analysis (**Figure 1**), no specific cut off was identified with high sensitivity and specificity for predicting HiSCR and IHS4 response in PIONEER 2 (the only study in which BMI was a significant covariate). The area under the ROC curve (AUC) was poor in the analysis with all subjects and by treatment arm (0.58–0.66).

TABLE 2 | (A) Association of BMI with treatment efficacy categorical variables and (B) Association of BMI with treatment efficacy continuous variables (Normal weight is the reference group).

(A)	**PIONEER 1**						**PIONEER 2**					
BMI variable	**Achieving HiSCR**		***P*-value**	**Achieving IHS4 category change**		***P*-value**	**Achieving HiSCR**		***P*-value**	**Achieving IHS4 category change**		***P*-value**
	Yes ($n = 102$)	No ($n = 187$)		Yes ($n = 92$)	No ($n = 197$)		Yes ($n = 134$)	No ($n = 155$)		Yes ($n = 115$)	No ($n = 174$)	
BMI (continuous)	33.9 ± 8.3	33.7 ± 7.7	0.84	35.0 ± 8.0	33.2 ± 7.8	0.07	29.9 ± 6.6	32.6 ± 6.4	**<0.001**	30.0 ± 6.3	32.2 ± 6.7	**0.005**
BMI underweight/normal	15 (41.7%)	21 (58.3%)	0.50	9 (25.0%)	27 (75.0%)	0.34	36 (63.2%)	21 (36.8%)	**0.01**	26 (45.6%)	31 (54.4%)	**0.03**
BMI overweight	20 (30.3%)	46 (69.7%)		18 (27.3%)	48 (72.7%)		33 (45.8%)	39 (54.2%)		36 (50.0%)	36 (50.0%)	
BMI obese	67 (35.8%)	120 (64.2%)		65 (34.8%)	122 (65.2%)		65 (40.6%)	95 (59.4%)		53 (33.1%)	107 (66.9%)	

(B)	**PIONEER 1**				**PIONEER 2**			
BMI variable	**%Change AN count**	***P*-value**	**%Change IHS4 count**	***P*-value**	**%Change AN count**	***P*-value**	**%Change IHS4 count**	***P*-value**
BMI underweight/normal	−36.2 ± 47.7	0.40	−25.8 ± 63.8	0.86	−51.2 ± 53.1	**0.02**	−43.2 ± 64.1	**0.04**
BMI overweight	−17.9 ± 63.9		−18.5 ± 64.0		−37.6 ± 59.1		−32.3 ± 60.5	
BMI obese	−23.2 ± 69.1		−21.6 ± 64.2		−25.4 ± 62.7		−17.7 ± 72.0	

Bold value represents BMI, Body mass Index; HiSCR, Hidradenitis Suppurativa Clinical Response; IHS4, International Hidradenitis Suppurativa Scoring System.

TABLE 3 | Results of logistic regression models of HiSCR achievement (Model 1) and IHS4 category change (Model 2) in patients treated with Adalimumab and Placebo in PIONEER 1 and PIONEER 2.

Variable	PIONEER 1 Achieving HiSCR			PIONEER 2 Achieving HiSCR		
Model 1	**Odds ratio**	**95 % CI**	***P*-value**	**Odds ratio**	**95 % CI**	***P*-value**
Adalimumab	**2.02**	**(1.22, 3.38)**	**0.007**	**4.20**	**(2.50, 7.24)**	**<0.001**
Hurley stage 3	0.90	(0.51, 1.57)	0.70	0.63	(0.36, 1.09)	0.10
Family history	0.76	(0.41, 1.39)	0.39	0.70	(0.38, 1.27)	0.24
Current smoker	0.98	(0.59, 1.65)	0.95	0.57	(0.32, 1.01)	0.06
Presence of draining tunnels	0.65	(0.34, 1.21)	0.17	**0.49**	**(0.27, 0.87)**	**0.02**
Antibiotic use	–	–	–	**0.45**	**(0.22, 0.90)**	**0.03**
BMI (overweight)	0.74	(0.31, 1.78)	0.49	**0.42**	**(0.19, 0.91)**	**0.03**
BMI (obese)	0.88	(0.41, 1.92)	0.75	**0.33**	**(0.16, 0.67)**	**0.002**
Male sex	0.87	(0.50, 1.51)	0.62	0.98	(0.54, 1.77)	0.95
Age	1.00	(0.97, 1.02)	0.74	1.00	(0.98, 1.02)	0.95
Variable	**PIONEER 1 achieving IHS4 category change**			**PIONEER 2 achieving IHS4 category change**		
Model 2	**Odds ratio**	**95 % CI**	***P*-value**	**Odds ratio**	**95 % CI**	***P*-value**
Adalimumab	**1.67**	**(0.99, 2.83)**	**0.05**	**2.89**	**(1.74, 4.88)**	**<0.001**
Hurley stage 3	**0.51**	**(0.29, 0.86)**	**0.01**	**0.57**	**(0.33, 0.95)**	**0.03**
Family history	1.01	(0.54, 1.86)	0.96	1.28	(0.72, 2.28)	0.40
Current smoker	0.79	(0.46, 1.33)	0.37	1.03	(0.60, 1.80)	0.91
Antibiotic use	–	–	–	0.72	(0.36, 1.38)	0.32
BMI (overweight)	1.35	(0.52, 3.67)	0.54	1.30	(0.62, 2.75)	0.49
BMI (obese)	1.50	(0.66, 3.67)	0.34	0.63	(0.32, 1.22)	0.17
Male sex	0.72	(0.40, 1.27)	0.26	0.58	(0.33, 1.02)	0.06
Age	1.00	(0.98, 1.02)	0.99	0.99	(0.97, 1.02)	0.64

Bold value represents BMI, Body mass Index; HiSCR, Hidradenitis Suppurativa Clinical Response; IHS4, International Hidradenitis Suppurativa Scoring System.

DISCUSSION

HS is a heterogenous disease (13, 14), but the relationship between this heterogeneity and BMI is incompletely defined. BMI is significantly associated with achieving HiSCR regardless of the status of BMI as a categorical or continuous variable. The reduction in odds is greater for participants in the obese category than the overweight category suggesting the accuracy of the general assumption that increasing BMI pre-disposes to a decrease in the efficacy of Adalimumab as measured by achieving HiSCR (9). BMI was significantly associated with change in AN count in univariate analysis (**Table 2**) in Pioneer 2 but was not significant when other variables including aspects of disease severity were taken into account (**Table 4**). It appears that the significant association of BMI in clinical response to Adalimumab is restricted to outcomes assessing a proportional rather than an absolute change in disease activity. This suggests that the association may be a product of the outcome measure rather than a true association; and may be more likely in those with lower baseline disease activity given that proportional change is inherently greater in the setting of lower baseline values.

This hypothesis would agree with what is observed with the difference in regression analyses between the PIONEER 1 and 2 studies (**Tables 3**, **4**). Disease severity (as measured by AN count and draining tunnel count) is significantly greater in PIONEER 1 than PIONEER 2 (**Supplementary Table 1**). Additionally, the median BMI for the PIONEER 1 cohort was significantly higher than the median BMI for the PIONEER 2 cohort (32.5 vs. 31.2 $p < 0.001$ by the Mann-Whitney *U*-Test) (**Supplementary Table 1**). This difference in BMI is equivalent to a 4% reduction in absolute weight (given an unchanging height) which is reported within the range of clinically significant weight change (15). In order to explore these observations further we examined the correlation between BMI and baseline disease activity (as measured by number of nodules/abscesses/AN count/number of draining tunnels) (**Supplementary Figure 1**). These results supported the findings of previous reports (2, 3, 16, 17) that BMI is not significantly associated with baseline number of nodules/abscesses/AN count/number of draining tunnels/IHS4 score although it is related to other outcomes such as the Sartorius score (16, 17). BMI accounts for <2.89% of the variation in baseline disease activity in PIONEER 2 across all measured examined (**Supplementary Figure 1**).

If significant correlation between BMI and baseline disease activity did exist, the significant findings in % change scores (HiSCR and % IHS4) compared to absolute changes (AN count and IHS4 category change) are logical. For a given dose of medication it is harder to achieve the same percentage change from a higher baseline than it is from a lower baseline. However,

TABLE 4 | Linear regression model of change in AN count (Model 1) and % change in IHS4 outcome measure in (Model 2) in Adalimumab treated patients in PIONEER 1 and PIONEER 2.

Variable	PIONEER 1 change in an count			PIONEER 2 change in an count		
Model 1	**Estimate**	**95 % CI**	***P*-value**	**Estimate**	**95 % CI**	***P*-value**
Adalimumab	**−2.26**	**(−4.31, −0.21)**	**0.03**	**−2.56**	**(−3.95, −1.16)**	**<0.001**
Hurley stage 3	**2.36**	**(0.12, 4.60)**	**0.04**	−0.18	(−1.64, 1.28)	0.81
Family history	−0.73	(−3.19, 1.73)	0.56	−0.58	(−2.17, 1.01)	0.47
Current smoker	−0.88	(−2.97, 1.21)	0.41	−0.02	(−1.54, 1.49)	0.98
Presence of draining tunnels	1.02	(−1.57, 3.61)	0.44	**1.88**	**(0.32, 3.44)**	**0.02**
Antibiotic use	–	–	–	1.10	(−0.68, 2.87)	0.22
BMI (overweight)	0.44	(−3.14, 4.02)	0.81	−0.13	(−2.22, 1.96)	0.90
BMI (obese)	0.21	(−2.97, 3.39)	0.90	0.11	(−1.73, 1.95)	0.91
Male sex	−0.41	(−2.65, 1.83)	0.72	0.05	(−1.52, 1.62)	0.95
Age	0.03	(−0.06, 0.12)	0.55	0.03	(−0.04, 0.09)	0.40
Variable	**PIONEER 1 % change in IHS4**			**PIONEER 2 % change in IHS4**		
Model 2	**Estimate**	**95 % CI**	***P*-value**	**Estimate**	**95 % CI**	***P*-value**
Adalimumab	**−18.70**	**(−33.81, −3.58)**	**0.02**	**−39.94**	**(−55.17, −24.71)**	**<0.001**
Hurley stage 3	8.31	(−6.99, 23.60)	0.29	2.79	(−12.67, 18.25)	0.72
Family history	−3.67	(−21.62, 14.28)	0.69	−5.97	(−23.35, 11.41)	0.50
Current smoker	1.13	(−14.22, 16.49)	0.88	4.75	(−11.78, 21.29)	0.57
Antibiotic use	–	–	–	18.14	(−1.22, 37.50)	0.07
BMI (overweight)	1.13	(−25.17, 27.43)	0.93	9.49	(−13.35, 32.33)	0.41
BMI (obese)	2.88	(−20.42, 26.17)	0.81	**22.61**	**(2.48, 42.75)**	**0.03**
Male sex	10.56	(−5.79, 26.92)	0.20	6.79	(−9.72, 23.30)	0.42
Age	0.28	(−0.39, 0.94)	0.42	−0.07	(−0.76, 0.62)	0.84

Bold value represents BMI, Body mass Index; HiSCR, Hidradenitis Suppurativa Clinical Response; IHS4, International Hidradenitis Suppurativa Scoring System.

given the presented data, one must conclude that an alternate reason for the discrepancy exists. The lack of association of BMI with disease activity and treatment response in PIONEER 1 as opposed to PIONEER 2 (**Tables 3**, **4**) raises the prospect that BMI may only demonstrate a significant association with response to Adalimumab in a subpopulation of participants with HS. This supports the previously reported concept of disease and pathogenic heterogeneity in HS (2, 17). Given the differences between the two PIONEER studies it may be hypothesized that the characteristics of the PIONEER 2 cohort are more reflective of the subpopulation (moderate disease, primarily nodules, low number of draining tunnels) where BMI demonstrates a significant association with clinical response to Adalimumab therapy.

In order to investigate this hypothesis, we analyzed the interaction between draining tunnels and BMI (both being significant covariates) as well as the contribution of baseline AN count in the linear and logistic regression models previously presented (**Tables 3**, **4**). No significant interaction effect was noted between BMI and the presence or absence of draining tunnels. However, when baseline AN count was introduced as a covariate it was (as expected) significantly associated with absolute change in AN count [$b = -0.37$ (95%CI $= -0.44, -0.30$) $p < 0.0001$] and IHS4 Category change [OR $=$ 0.94 (95%CI $=$ 0.90, 0.97), $p = 0.002$] in PIONEER 2. The significance of BMI was not altered with the addition of the baseline AN covariate into the regression model, although Hurley stage became non-significant in the logistic regression model of achieving IHS4 category change (**Supplementary Table 2**). One critique of examining BMI by categorical variables is that traditional BMI cut-offs are assumed to be best suited to understanding the relationship between BMI and HS disease severity scores. However, in order to not rely upon this assumption, we examined this relationship using ROC, sensitivity and specificity analyses, and no alternative cut off was identified to further examine this relationship.

Overall, these in-depth analyses suggest that the significant association of BMI in the response to Adalimumab is maintained when other factors of baseline disease activity are taken into account. This supports the concept that BMI plays a significant independent role in disease response to Adalimumab therapy, however this significance may only be present in a subpopulation of individuals with HS given the discrepancies between regression models and PIONEER 1 and 2 cohort characteristics.

The findings of this analysis can be used to direct further mechanistic enquiry into the molecular pathogenesis of HS. Adipose tissue derived pro-inflammatory mediators (such as polyunsaturated fatty acids and the lipoxygenase pathway) (18) have been demonstrated to be significantly elevated in lesional HS tissue (nodules) as well as in individuals with elevated BMI (19)

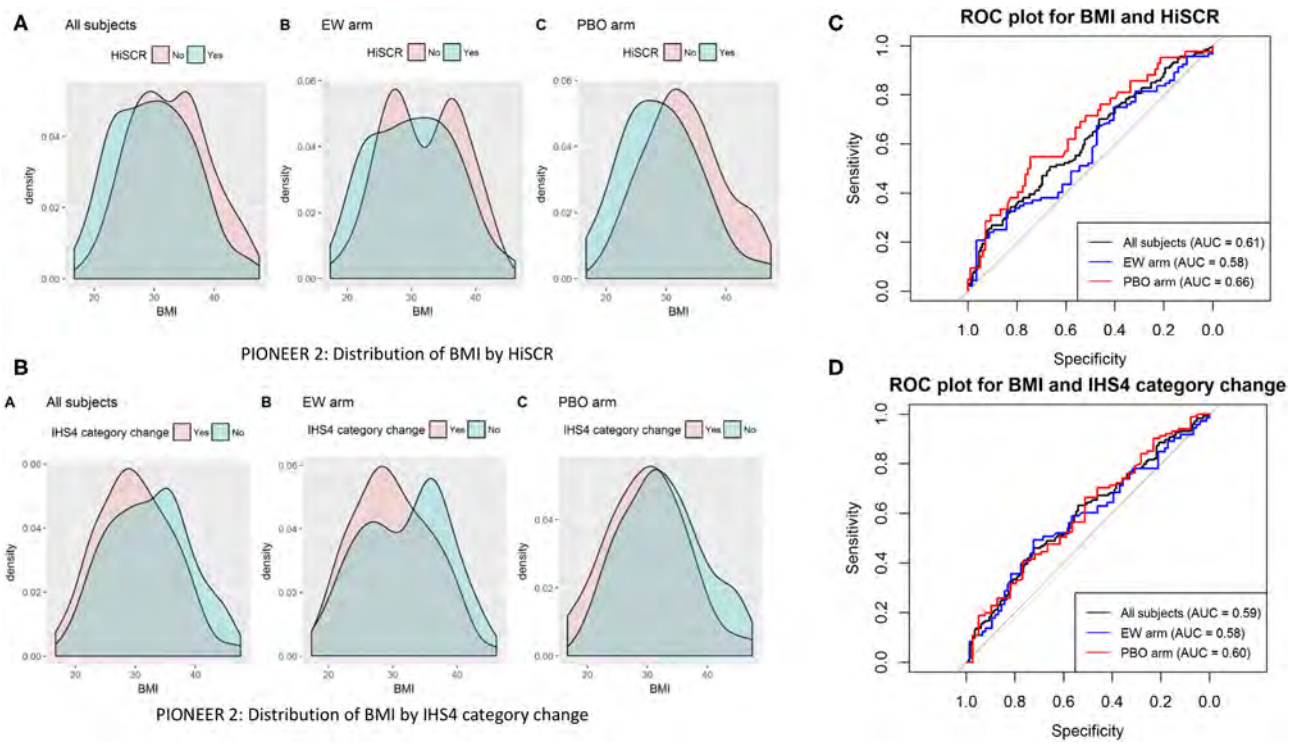

FIGURE 1 | Distribution **(A,B)** and ROC plots **(C,D)**. Receiver operating characteristic (ROC) curves of BMI against HiSCR and IHS4 category change.

compared to healthy controls (18). These observations suggest that such mediators may have a role in the initiation phase (20) of clinical disease in HS (associated with reduced number of lesions) rather than activity in severe established disease. This hypothesis would be supported by the evidence surrounding bariatric surgery and weight loss in the treatment of HS (21–23), with reports of excellent response only in Hurley stage 1 and 2 patients (21–23).

Limitations to this study include the inherent limitations of using clinical trial data, including acknowledging the limitations in extrapolating this data to the wider HS population (24).

Based upon the body weights of participants in this study, the range of dosages (mg/kg) for Adalimumab only ranged between 0.26 and 0.93 mg/kg and hence any extrapolation beyond this range of dosages is inaccurate. In addition, the potential mechanistic links between BMI and nodules require further investigations in molecular, mechanistic and translational studies.

BMI is significantly associated with response to Adalimumab in the treatment of HS as measured by HiSCR. An increase in BMI was associated with decreased odds of achieving HiSCR in the PIONEER 2 study. BMI was not significantly associated with IHS4 category change or change in AN count; but percentage change in IHS4 score was significantly attenuated in participants with obese BMI. The discrepancies between the association of BMI and treatment response may be explained by BMI only being a significant influence in a subpopulation of participants with lower baseline AN count. Therefore, further mechanistic studies are needed to reliably identify this subpopulation in which BMI has a significant association with response to Adalimumab and evaluate the role of increases doses of Adalimumab or weight loss interventions in this cohort.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: This publication is based upon research data from Abbvie Inc. Abbvie Inc. had no input into the design or execution of the study, statistical analysis or composition of the article. Requests to access these datasets should be directed to Vivli Inc. support@vivli.org.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by this study is a re-analysis of de-identified patient data provided by data contributor Abbvie Inc. through Vivli Inc. This re-analysis was approved by the Institutional Review Board of the Rockefeller University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JF and RV designed the study. JF, NS, and CJ understood the methodology and statistical analysis. JK supervised

the project. JF wrote the initial manuscript. All authors contributed to revisions and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.603281/full#supplementary-material

Supplementary Figure 1 | Scatterplots of the relationships between BMI and aspects of disease activity in HS for PIONEER 1 **(A)** and PIONEER 2 **(B)**.

Supplementary Table 1 | Table comparing the demographic and disease specific characteristics of participants in PIONEER 1 and PIONEER 2.

Supplementary Table 2 | Regression Models with Baseline AN Count as a significant covariate in achieving IHS4 category change (Model 3) and change in AN count (Model 4) in PIONEER 2.

REFERENCES

1. Nguyen TV, Damiani G, Orenstein LAV, Hamzavi I, Jemec GB. Hidradenitis suppurativa: an update on epidemiology, phenotypes, diagnosis, pathogenesis, comorbidities and quality of life. *J Eur Acad Dermatol Venereol*. (2021) 35:50–61.
2. Kromann CB, Ibler KS, Kristiansen VB, Jemec GB. The influence of body weight on the prevalence and severity of hidradenitis suppurativa. *Acta Derm Venerol*. (2014) 94:552–7. doi: 10.2340/00015555-1800
3. Crowley JJ, Mekkes JR, Zouboulis CC, Scheinfeld N, Kimball A, Sundaram M, et al. Association of hidradenitis suppurativa disease severity with increased risk for systemic comorbidities. *Br J Dermatol*. (2014) 171:1561–5. doi: 10.1111/bjd.13122
4. Alikhan A, Sayed C, Alavi A, Alhusayen R, Brtassard A, Burkhart C, et al. North American clinical management guidelines for hidradenitis suppurativa: a publication from the United States and Canadian Hidradenitis Suppurativa Foundations. *J Am Acad Dermatol*. (2019).
5. Kimball AB, Okun MM, Williams DA, Gottleib AB, Papp KA, Zouboulis CC, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. *N Engl J Med*. (2016) 375:422–34. doi: 10.1056/NEJMoa1504370
6. Ingram JR. Interventions for hidradenitis suppurativa: updated summary of an original cochrane review. *JAMA Dermatol*. (2017) 153:458–9. doi: 10.1001/jamadermatol.2017.0432
7. Ashley N, Oskardmay BS, Miles JA, Sayed CJ. Determining the optimal dose of infliximab for treatment of hidradenitis suppurativa. *J Am Acad Dermatol*. (2019) 81:702–8. doi: 10.1016/j.jaad.2019.05.022
8. Kimball AB, Jemec GBE, Yang M, Kageleiry A, Signorovitch JE, Okun MM, et al. Assessing the validity, responsiveness and meaningfulness of the hidradenitis suppurativa clinical response (HiSCR) as the clinical endpoint for hidradenitis suppurativa treatment. *Br J Dermatol*. (2014) 171:1434–42. doi: 10.1111/bjd.13270
9. Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, et al. Clinical response rates, placebo response rates and significant associated covariates are dependent upon choice of outcome measure in hidradenitis suppurativa. A *post-hoc* analysis of PIONEER 1 and PIONEER 2 individual patient data. *J Am Acad Dermatol*. (2020) 82:1150–7. doi: 10.1016/j.jaad.2019.12.044
10. Theut Riis P, Saunte D, Benhadou F, del Marmol V, Guillem P, El-Domyati M, et al. Low and high body mass index in hidradenitis suppurativa patients-different subtypes? *J Eur Acad Dermatol Venereol*. (2018) 32:307–12. doi: 10.1111/jdv.14599
11. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975-2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet*. (2016) 387:1377–96. doi: 10.1016/S0140-6736(16)30054-X
12. R Core Team (2017). *R: A Language and Environment for Statistical Computing*. R Project for Statistical Computing, Vienna, Austria. Available online at: https://www.R-project.org/ (accessed May 23, 2021).
13. Van der Zee HH, Jemec GBE. New insights into the diagnosis of hidradenitis suppurativa: clinical presentations and phenotypes. *J Am Acad Dermatol*. (2015) 73(Supp. 1):S23–26. doi: 10.1016/j.jaad.2015.07.047
14. Navrazhina K, Garcet S, Gonzalez J, Grand D, Frew JW, Krueger J. In depth analysis of the hidradenitis suppurativa proteome identified distinct inflammatory subtypes. *J Invest Dermatol*. (2021). doi: 10.1016/j.jid.2021.02.742
15. Jensen MD, Ryan DH, Apovian CM. A report of the American College of Cardiology/American Heart Association task force on practice guidelines and the obesity society. *Circulation*. (2014) 129:S102–38. doi: 10.1161/01.cir.0000437739.71477.ee
16. Fabbrocini G, De Vita V, Donnarumma M, Russo G, Monfrecola G. South Italy: a provileged perspective to understand the relationship between hidradenitis suppurativa and overweight/obesity. *Skin Appendage Disord*. (2016) 2:52–6. doi: 10.1159/000447716
17. Vazquez BG, Alikhan A, Weaver AL, Wetter DA, Davis MD. Incidence of hidradenitis suppurativa and associated factors: a population-based study of Olmsted county, Minnesota. *J Invest Dermatol*. (2013) 133:97–103. doi: 10.1038/jid.2012.255
18. Penno CA, Jager P, Laguerre C, Hasler F, Hofmann A, Gass S. Lipidomic profiling of hidradenitis suppurativa skin lesions reveals lipoxygenase pathway dysregulation and accumulation of pro-inflammatory leukotriene B4. *J Invest Dermatol*. (2020) 140:2421–2432.e10. doi: 10.1016/j.jid.2020.04.011
19. Mousa A, Naderpoor N, Mellett N, Wilson K, Plebanski M, Meikle PJ, et al. Lipidomic profiling reveals early-stage metabolic dysfunction in overweight or obese humans. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2019) 1864:335–43. doi: 10.1016/j.bbalip.2018.12.014
20. Hoffman LK, Ghias MH, Lowes MA. Pathophysiology of hidradenitis suppurativa. *Semin Cutan Med Surg*. (2017) 36:47–54. doi: 10.12788/j.sder.2017.017
21. Gallagher C, Kirthi S, Burke T, O'Shea D, Tobin AM. Remission of hidradenitis suppurativa after bariatric surgery. *JAAD Case Rep*. (2017) 3:436–7. doi: 10.1016/j.jdcr.2017.06.008
22. Thomas CL, Gordon KD, Mortimer PS. Rapid resolution of hidradenitis suppurativa after bariatric surgical intervention. *Clin Exp Dermatol*. (2014) 39:315–7. doi: 10.1111/ced.12269
23. Boer J. Resolution of hidradenitis suppurativa after weight loss by dietary measures, especially on frictional locations. *J Eur Acad Dermatol Venereol*. (2016) 30:895–6. doi: 10.1111/jdv.13059

24. Ingram JR, Piguet V. Phenotypic heterogeneity in hidradenitis suppurativa (acne inversa): classification is an essential step toward personalized therapy. *J Invest Dermatol*. (2013) 133:1453–6. doi: 10.1038/jid.2012.476

Conflict of Interest: JK has received research support (grants paid to institution) from AbbVie, Amgen, BMS, Boehringer, EMD Serono, Innovaderm, Kineta, LEO Pharma, Novan, Novartis, Paraxel, Pfizer, Regeneron, and Vitae and personal fees from AbbVie, Acros, Allergan, Aurigne, BiogenIdec, Boehringer, Escalier, Janssen, Lilly, Novartis, Pfizer, Roche, and Valeant.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.4.3: Publication 4-3

Frew JW, Jiang C, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger J

"Malignancy and Infection Risk During Adalimumab Therapy in Hidradenitis Suppurativa" Clin Exp Dermatol 2020; 45(7):859-865

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https://onlinelibrary.wiley.com/doi/10.1111/ced.14264

4.5: Translational and Clinical Trial Methodology in HS:

Manuscript	Manuscript Reference
5-1	Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR et al Defining Lesional, Perilesional and Unaffected Skin in Hidradenitis Suppurativa: Proposed Recommendations for Clinical Trials and Translational Research Studies Br J Dermatol 2019; 181(6):1339-1341
5-2	Frew JW, Jiang C, Singh N, Grand D, Navrazhina K Vaughan R, Krueger JG Clinical Response Rates, Placebo Response Rates and Significantly Associated Covariates Are Dependent Upon Choice of Outcome Measure in Hidradenitis Suppurativa: A Post-Hoc Analysis of PIONEER 1 and 2 Individual Patient Data J Am Acad Dermatol 2019; 82(5):1150-1157
5-3	Frew JW "Primary Imputation Methods Impact Efficacy Results in Hidradenitis Suppurativa Clinical Trials" J Am Acad Dermatol 2020; 83(2):663-665
5-4	Frew JW, Jiang CS, Singh N, Navrazhina K, Vaughan R, Krueger JG Quantifying the Natural Variation in Lesion Counts over time in Untreated Hidradenitis Suppurativa: Implications for Outcome Measures and Trial Design. JAAD International 2020; 1(2):208-221

Table 4-5-1 Publications Presented in this Chapter pertaining to clinical trial design in HS

There is a wide range of variation in the sampling techniques used in investigational and translational trials in HS. There have been attempts to describe best practice[77] for the selection and isolation of tissue samples in HS, and global consensus is an important step towards achieve comparable results across investigations and an increased understanding of the pathogenesis of disease.

The first publication in this chapter (5-1) provides recommendations regarding tissue biopsy techniques for translational and investigative studies in HS. The complex morphology of the disease and the presence of occult tunnels with significant inflammatory potential (See Chapter 4.2) make consistency across studies a vital step

in understanding the disease. The publication was co-authored by a number of experts in the field with the recommendation of formal consensus amongst all key stakeholders in the future including those companies developing and conducting clinical trials with biomarker development programs.

Regarding clinical trials, recent years have seen a total of four failed Phase 2 clinical trials in Hidradenitis Suppurativa. A major concern in these trials which have led to the failure to achieve primary outcome has been the high levels of placebo response rates when HISCR has been the primary outcome measure[78]. In order to examine the contribution of the outcome measure to placebo response rates, individual patient data from the Phase 3 studies of Adalimumab in HS were analysed. An alternate proposed outcome measure – the IHS4 – was calculated and placebo response rates calculated, along with relevant covariates which impact clinical response rates. Major findings include that the HiSCR itself is a major contributor to placebo response rates and that placebo response rates were lower when continuous variables were used as primary outcome measures. Additionally, factors such as the presence of epithelialised tunnels and increasing BMI has an association with clinical response as measured by HiSCR. Given that a total of four major Phase 2 clinical trial programs for HS (IFX-1, Avacopan, Guselkumab, Risankizumab) have now failed to achieve primary outcomes due to high (30-49%) placebo response rates- this evidence of alternate outcome measures is vital to the appropriate design of future trials.

The third included publication focuses upon another aspect of clinical trial design- primary imputation methods. Whilst the single Phase 3 clinical trial in HS utilised a Non-Responder Imputation design, many smaller scale clinical trials and proof of concept studies, as well as the open label extension study of Adalimumab in HS, utilised last observation carried forward analysis. Whilst LOCF can be appropriate for endpoints which are permanent (death, recurrence of malignancy), for HS where clinical outcomes can wax and wane, it may overestimate clinical response. The major findings of this analysis show that in various agents, LOCF are erroneously used which may overestimate clinical response.

The fourth and final publication in this chapter pertains to the quantification of the natural variability of lesion counts in the setting of untreated HS. This is an important aspect which has not previously been quantified. Given the highly variable and transient nature of individual lesions in the disease, any outcome measures which rely upon serial measurement of disease activity (ie- lesion counts over time), need to have a reliable baseline for lesion variability. This would inform inclusion and exclusion criteria to prevent elevation of placebo response rates through individuals achieving HiSCR through a reduction in 1 or 2 nodules unrelated to the exposure of interest.

The major findings of this publication were that the natural baseline variability of untreated disease was 33% over a 12-week period (for nodules specifically). Alternatives for reducing placebo response rates in future trials would include increasing inclusion criteria of future trials to those with >7 inflammatory nodules; setting clinical response outcome measures to a 75% reduction in lesion counts, or abandoning counting of lesions in favour of an alternate outcome measure such as the HASI[79,80].

Since publication, many clinical trials have begun to alter inclusion criteria to >5 inflammatory nodules in order to reduce placebo response rates. Additionally, the IHS4[79] and HASI[80] are both commonly integrated into clinical trials in the assessment of novel therapeutics. The HASI is a novel endpoint not based upon counting lesions, but rather body surface area calculations and will provide another novel clinical trial endpoint[80].

4.5.1: Publication 5-1

Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR et al Defining Lesional, Perilesional and Unaffected Skin in Hidradenitis Suppurativa: Proposed Recommendations for Clinical Trials and Translational Research Studies Br J Dermatol 2019; 181(6):1339-1341

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4.5.2: Publication 5-2

Frew JW, Jiang C, Singh N, Grand D, Navrazhina K Vaughan R, Krueger JG Clinical Response Rates, Placebo Response Rates and Significantly Associated Covariates Are Dependent Upon Choice of Outcome Measure in Hidradenitis Suppurativa: A Post-Hoc Analysis of PIONEER 1 and 2 Individual Patient Data J Am Acad Dermatol 2019; 82(5):1150-1157

Clinical response rates, placebo response rates, and significantly associated covariates are dependent on choice of outcome measure in hidradenitis suppurativa: A post hoc analysis of PIONEER 1 and 2 individual patient data

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Background: The hidradenitis suppurativa clinical response (HiSCR) is the gold standard primary outcome measure for hidradenitis suppurativa clinical trials; however, it does not assess the presence of draining tunnels, a common finding in advanced disease. It is unclear what the effect of the presence or absence of draining tunnels has on the efficacy of adalimumab therapy in moderate and advanced disease.

Objectives: We evaluated the efficacy of adalimumab versus placebo using the International Hidradenitis Suppurativa Severity Scoring System (IHS4). Additionally, we assessed the effect of draining tunnels on therapeutic response as measured by both the HiSCR and change in nodule counts.

Methods: Reanalysis was conducted with the IHS4 and PIONEER 1 and 2 individual patient data. Both binary outcomes (achieving HiSCR and achieving change in IHS4 severity category) and continuous outcomes (nodule counts and IHS4 score) were calculated with R. Regression modeling was undertaken to assess the effect of draining tunnels and other variables. $P < .05$ was considered statistically significant.

Results: The significance of adalimumab therapy depended on the outcome measure used. Placebo response rates were highest when binary outcome measures were used. Draining tunnels, smoking, antibiotics, and body mass index influenced HiSCR response in PIONEER 2. Significant differences in disease severity were observed between PIONEER 1 and 2 data sets.

Conclusions: Elevated placebo response rates in PIONEER 1 and 2 are partially attributable to the use of binary outcome measures. Draining tunnels influence clinical response as measured by HiSCR and nodule

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counts in PIONEER 2. Further investigation into the effect of body mass index on clinical response is required. (J Am Acad Dermatol 2020;82:1150-7.)

Key words: hidradenitis suppurativa; acne inversa; HiSCR; IHS4; outcome measures; BMI; tunnels; placebo.

BACKGROUND

The hidradenitis suppurativa clinical response (HiSCR)[1] outcome measure is currently considered the gold standard primary outcome measure for the assessment of new pharmacologic interventions in hidradenitis suppurativa clinical trials.[1,2] HiSCR is defined as a 50% reduction in abscess and nodule count without any increase in the number of abscesses or draining tunnels relative to baseline.[1] However, high rates of placebo response have been identified and are problematic for the evaluation of novel pharmacologic interventions in this disease.[3] As such, studies using the HiSCR may be prone to measurement bias when comparing different stages and severities of disease.[4] The International Hidradenitis Suppurativa Severity Scoring System[5] (IHS4), developed by the European Hidradenitis Suppurativa Foundation Investigator Group, is an alternative outcome measure that is often included as a secondary outcome, but to our knowledge the results of this outcome measure have not been reported in any phase 3 clinical trial to date, nor have there been any attempts to compare different outcome measures using the same clinical trial data set. This comparison would enable the identification of specific clinical variables that may predict response to therapy and also allow the evaluation of measurement bias within specific outcome measures themselves.

Given the heterogeneous clinical manifestations of hidradenitis suppurativa[6] (including nodules, abscesses, tunnels, and scarring), the quantification of abscesses and nodules as an outcome measure (HiSCR) does not take into account the response of draining tunnels to pharmacologic therapy.

Given the overall response rates of hidradenitis suppurativa to adalimumab (41.8% and 58.9% in the PIONEER 1 and 2 studies, respectively)[7,8] and the significant dropout rates in existing studies because of lack of efficacy (27%-50%),[7,8] it is important that we understand the effect of draining tunnels on treatment efficacy.

We hypothesized that the presence of draining tunnels in hidradenitis suppurativa has no effect on rates of clinical response to adalimumab therapy. This was assessed through comparison of 2 outcome measures (HiSCR and IHS4, both as binary and continuous variables) within the PIONEER 1 and PIONEER 2 phase 3 clinical trial data set at week 12 compared with baseline (week 0).

Our specific aims included evaluating the efficacy of adalimumab versus placebo using the IHS4 outcome measure in place of HiSCR and assessing the effect of the presence of draining tunnels on clinical response as measured by the HiSCR and change in nodule counts.

CAPSULE SUMMARY

- The hidradenitis suppurativa clinical response clinical end point is the gold standard outcome measure in hidradenitis suppurativa clinical trials. The effect of draining tunnels (in advanced disease) on the measured efficacy of adalimumab in hidradenitis suppurativa is not well described. Other outcome measures (such as the International Hidradenitis Suppurativa Severity Scoring System) include draining tunnels, but no direct comparison of outcome measures within a common data set has been undertaken.
- Clinical response to adalimumab is significantly greater than placebo regardless of the use of outcome measure in PIONEER 2 but not PIONEER 1. Placebo response rates in the PIONEER 1 and 2 phase 3 trials are significantly lower when the hidradenitis suppurativa clinical response is replaced by the International Hidradenitis Suppurativa Severity Score System. Draining tunnels, smoking, antibiotic use, and body mass index are significantly associated with reduced hidradenitis suppurativa clinical response in PIONEER 2, and differences between results of PIONEER 1 and 2 studies are attributable to different disease severities of patient populations.

METHODS

Deidentified individual patient data from PIONEER 1 and 2 studies were made available by AbbVie Inc and accessed through the secure Vivli online platform.[7] Raw data were extracted and compared with the available published data to ensure accuracy.[7] Only data for period A (week 0 to week 12) comparing adalimumab 40 mg weekly versus placebo were included in the analysis to

Abbreviations used:

BMI: body mass index
CI: confidence interval
HiSCR: hidradenitis suppurativa clinical response
IHS4: International Hidradenitis Suppurativa Severity Scoring System
OR: odds ratio

reflect current dosing recommendations. Individuals with incomplete data and those who received antibiotic therapy in PIONEER 1 were excluded from analysis. Antibiotic therapy in PIONEER 2 was included as a covariate. Our statistical methods mirrored those of the PIONEER 1 and 2 statistical analysis,[7] with the exception that the HiSCR (sliding dichotomous variable) was replaced with the IHS4. The IHS4 was expressed as a continuous variable, using available raw individual patient data according to the published equation by Zouboulis et al[5] (nodule count) + (abscess count × 2) + (draining tunnel count × 4). It was also calculated as a sliding dichotomous variable determined by progression to a lower-severity category. Severity categories (mild 0-3; moderate 4-10; severe ≥11) were derived from Zouboulis et al.[5] All data analysis was conducted in R (version 3.5.3; R Core Team, Vienna, Austria).[9]

Each variable of interest was assessed for normality with the Shapiro-Wilk test and histograms. The differences between treatment groups were compared with Welch's *t* test for normally distributed continuous variables and the Mann-Whitney U test for nonnormally distributed continuous variables. Chi-square and Fisher's exact tests were used for categorical variables. Potential associations with the presence of draining tunnels, as well as other a priori potential associations (age, sex, Hurley stage, smoking status, family history, antibiotic use [for PIONEER 2 only], and body mass index [BMI]), were assessed with logistic regression for HiSCR and binary IHS4 and with linear regression for percentage change in IHS4 and absolute change in nodule count. Draining tunnels was not investigated as a covariate in linear or logistic expression when IHS4 was the outcome of interest (because draining tunnels were a component of the IHS4). $P < .05$ was considered statistically significant.

RESULTS

Demographic characteristics of the participants included in the statistical analysis are presented in Table I. The number, percentage, and intergroup differences between adalimumab and placebo arms, as measured by the HiSCR, change in IHS4 severity category, change in nodule counts, and percentage change in IHS4 score are presented in Table II. Statistically significant differences between adalimumab and placebo therapy were observed regardless of whether HiSCR, change in nodule counts, or change in IHS4 score was used as the primary outcome measure (Table II). Change in IHS4 severity category as an outcome measure identified statistically significant change only in PIONEER 2 (Table II). Rates of placebo response were markedly lower when continuous variables (as opposed to sliding dichotomous ones) were used as primary outcome measures (Table II).

Unadjusted logistic regression identified greater odds of HiSCR with adalimumab compared with placebo in PIONEER 1 (odds ratio [OR] 1.98; 95% confidence interval [CI] 1.22-3.26; $P = .006$). When adjusted for covariates, adalimumab therapy displayed greater odds than placebo of association with achieving HiSCR (OR 2.05; 95% CI 1.25-3.47; $P = .005$). No covariates were statistically significant in altering the odds of achieving HiSCR (Table III). Adalimumab had increased odds of association with an HiSCR response in unadjusted analysis of PIONEER 2 (OR 3.77; 95% CI 2.32-6.19; $P < .001$). When covariates were adjusted, patients receiving adalimumab had a further increase in the odds of achieving HiSCR than placebo (OR 4.22; 95% CI 2.50-7.28; $P < .001$). Current smokers had reduced odds of achieving HiSCR compared with nonsmokers (OR 0.56; 95% CI 0.31-0.98; $P = .04$), and the presence of draining tunnels reduced the odds of achieving HiSCR (OR 0.45; 95% CI 0.25-0.79; $P = .01$). In addition, the use of antibiotics reduced the odds of achieving HiSCR (OR 0.47; 95% CI 0.23-0.93; $P = .03$) and every unit increase in BMI significantly reduced the odds of achieving HiSCR by 7.1%. (OR 0.93; 95% CI 0.89-0.97; $P < .001$).

No significant difference in OR was identified between adalimumab and placebo in achieving IHS4 category change in PIONEER 1 (OR 1.58; 95% CI 0.96-2.62; $P = .07$). After adjusting for covariates, adalimumab still did not significantly increase the odds of achieving IHS4 category change versus placebo (OR 1.69; 95% CI 1.00-2.86; $P = .05$). Hurley stage 3 disease significantly reduced the odds of achieving IHS4 category change (OR 0.52; 95% CI 0.30-0.88; $P = .02$). Patients receiving adalimumab had increased odds of achieving IHS4 category change compared with those receiving placebo in PIONEER 2 (OR 2.70; 95% CI 1.66-4.43; $P = <.001$). Adjusting for covariates increased the overall odds (OR 2.91; 95% CI 1.75-4.91; $P = <.001$), with Hurley stage 3 disease (OR 0.57; 95% CI 0.33-0.95; $P = .03$), increase in BMI (OR 0.95; 95% CI 0.91-0.98; $P = .01$), and male sex (OR 0.55; 95% CI

Table I. Population characteristics

	PIONEER 1			PIONEER 2		
Characteristic	**Adalimumab**	**Placebo**	***P* value**	**Adalimumab**	**Placebo**	***P* value**
N	144	145		149	140	
Women	85 (59.0)	100 (69.0)	.10	97 (65.1)	98 (70.0)	.45
Men	59 (41.0)	45 (31.0)		52 (34.9)	42 (30.0)	
White	111 (77.1)	113 (77.9)	.35	130 (87.2)	110 (78.6)	.07
Black	30 (20.8)	25 (17.2)		8 (5.4)	18 (12.9)	
Other	3 (2.1)	7 (4.8)		11 (7.4)	12 (8.6)	
Age, y						
Median	35.0 (28.0, 45.0)	37.0 (30.0, 47.0)	.14	35.0 (27.0, 42.0)	35.0 (26.0, 43.3)	.49
Mean	36.5 ± 11.0	38.4 ± 11.4		34.8 ± 10.1	36.4 ± 12.2	
BMI						
Median	32.1 (27.1, 38.0)	33.9 (28.5, 39.4)	.07	30.3 (26.3, 36.0)	31.3 (26.8, 36.0)	.22
Mean	32.9 ± 7.7	34.6 ± 8.1		30.9 ± 6.4	31.8 ± 6.8	
Hurley stage						
2	80 (55.6)	79 (54.5)	.95	76 (51.0)	79 (56.4)	.42
3	64 (44.4)	66 (45.5)		73 (49.0)	61 (43.6)	
Nicotine use	77 (53.5)	88 (60.7)	.26	96 (64.4)	99 (70.7)	.31
Family history	37 (25.7)	28 (19.3)	.25	36 (24.2)	39 (27.9)	.56
Presence of draining tunnels	108 (75.0)	108 (74.5)	>.99	99 (66.4)	87 (62.1)	.52
Antibiotics	—	—		27 (18.1)	28 (20.0)	.80
Nodules						
Median	8 (4.75, 14)	7 (4, 15)	.88	6 (4,11)	6 (4, 10.25)	.98
Mean	11.4 ± 11.1	11.6 ± 14.2		8.2 ± 6.0	8.8 ± 8.0	
Abscesses						
Median	1.5 (0, 4)	2 (0, 3)	.77	1 (0, 3)	1 (0, 3)	.88
Mean	2.7 ± 3.3	2.6 ± 3.6		2.0 ± 2.5	2.3 (3.2)	
Draining tunnels						
Median	2.5 (0.75, 7)	2 (0, 5)	.38	2 (0, 4)	1 (0, 4)	.60
Mean	4.5 ± 5.1	3.7 ± 4.3		3.0 ± 4.0	3.5 ± 5.8	
Baseline IHS4						
Median	26.5 (15, 45.25)	25.0 (12, 40)	.28	19 (10, 34)	18 (8.75, 32.25)	.91
Mean	34.7 ± 26.8	31.6 ± 27.9		24.2 ± 20.0	27.3 ± 29.3	

Data are reported as no. (%) with median (25th and 75th percentile) and mean ± standard deviation for age, BMI, nodules, abscesses, draining tunnel counts, and baseline IHS4. *P* values were calculated with the χ^2 or Fisher's exact test for categorical variables, Mann-Whitney U test for non-normally distributed continuous data, and Welch's *t* test for normally distributed continuous data. *BMI*, Body mass index; *IHS4*, International Hidradenitis Suppurativa Severity Score; —, not applicable.

0.31-0.96; $P = .04$) significantly reducing the odds of IHS4 category change.

Linear regression identified adalimumab therapy as associated with a mean alteration in nodule count of 2 at week 12 compared with placebo (b = −2.38; 95% CI −4.38 to −0.38; $P = .02$) in PIONEER 1. Accounting for covariates, the association with adalimumab remained significant, implying that, all other covariables being the same, the mean change in nodule count was on average higher by 2 nodules for patients with Hurley stage 3 at week 12 compared with stage 2 (b = 2.23; 95% CI 0.01-4.48; $P = .05$). PIONEER 2 demonstrated a degree of alteration in mean nodule count similar to that of adalimumab therapy in unadjusted analysis (b = −2.54; 95% CI −3.92 to −1.16; $P < .001$) and adjusted analysis (b = −2.58; 95% CI −3.97 to −1.19; $P < .001$). The mean change in nodule count was on average higher at week 12 in the presence of draining tunnels (b = 1.87; 95% CI 0.32-3.43; $P = .02$) compared with the absence of them.

Linear regression identified that adalimumab therapy was associated with an average reduction of 18.74% in IHS4 compared with placebo in unadjusted analysis of PIONEER 1 (b = −18.74; 95% CI −32.97 to −4.57; $P = .01$). With inclusion of covariates, adalimumab treatment was significantly associated with an 18.60% reduction in IHS4 compared with placebo (b = −18.60; 95% CI −33.64 to −3.55; $P = .02$) (Table IV). Unadjusted analysis of PIONEER 2 illustrated a 41.11% reduction in IHS4 with adalimumab compared with placebo (b = −41.11; 95% CI −56.23 to −25.99; $P < .001$). In PIONEER 2, adalimumab therapy was associated

Table II. Comparing hidradenitis suppurativa clinical response and International Hidradenitis Suppurativa Severity Score (as both binary and continuous variables) as primary outcome measures in PIONEER 1 and 2 phase 3 randomized controlled trial data

	PIONEER 1			PIONEER 2		
Outcome measure at week 12	**Adalimumab**	**Placebo**	***P* value**	**Adalimumab**	**Placebo**	***P* value**
N	144	145		149	140	
No. of patients achieving HiSCR (%)	62 (43.06)	40 (27.59)	**.01**	92 (61.74)	42 (30.00)	**<.001**
No. of patients achieving change in IHS4 category (%)	53 (36.81)	39 (26.90)	.09	76 (51.01)	39 (27.86)	**<.001**
Mean change in AN counts (mean % change from baseline)	−5.47 (−33.80)	−2.81 (−13.51)	**.006**	−5.64 (−50.02)	−2.24 (−16.01)	**<.001**
Mean change in IHS4 value (mean % change from baseline)	−11.08 (−30.82)	−4.91 (−12.08)	**.002**	−10.36 (−46.29)	−1.33 (−5.18)	**<.001**

AN, total abscess and inflammatory nodule count; *HiSCR*, Hidradenitis suppurativa clinical response; *IHS4*, International Hidradenitis Suppurativa Severity Score; —, not applicable.
Bold data indicates statistical significance.

Table III. Results of logistic regression models of hidradenitis suppurativa clinical response achievement (model 1) and International Hidradenitis Suppurativa Severity Score category change (model 2) in patients treated with adalimumab and placebo in PIONEER 1 and 2

	PIONEER 1			PIONEER 2		
Variable	**Odds ratio**	**95% CI**	***P* value**	**Odds ratio**	**95% CI**	***P* value**
Model 1	Achieving HiSCR					
Adalimumab	**2.08**	**1.25−3.47**	**.005**	**4.23**	**2.51−7.31**	**<.001**
Hurley stage 3	0.91	0.52−1.59	.74	0.64	0.37−1.11	.11
Family history	0.77	0.41−1.40	.40	0.73	0.39−1.32	.30
Current smoker	0.98	0.59−1.65	.94	**0.56**	**0.32−1.0**	**.05**
Presence of draining tunnels	0.63	0.34−1.18	.15	**0.47**	**0.26−0.84**	**.01**
Antibiotic use	—	—	—	**0.48**	**0.24−0.95**	**.04**
BMI	1.01	0.97−1.04	.74	**0.93**	**0.89−0.97**	**<.001**
Male sex	0.85	0.49−1.47	.57	0.89	0.49−1.61	.70
Age	1.0	0.97−1.02	.73	1.0	0.98−1.02	.97
Model 2	Achieving IHS4 category change					
Adalimumab	**1.69**	**1.00−2.86**	**.05**	**2.91**	**1.75−4.92**	**<.001**
Hurley stage 3	**0.52**	**0.30−0.88**	**.02**	**0.57**	**0.33−0.95**	**.03**
Family history	1.02	0.55−1.86	.96	1.25	0.70−2.22	.45
Current smoker	0.82	0.48−1.39	.45	1.01	0.58−1.76	.97
Antibiotic use	—	—	—	0.76	0.39−1.47	.42
BMI	1.02	0.99−1.06	.22	**0.95**	**0.91−0.98**	**.006**
Male sex	0.73	0.41−1.29	.29	**0.55**	**0.31−0.96**	**.04**
Age	1.0	0.98−1.02	.94	0.99	0.97−1.02	.63

BMI, Body mass index; *CI*, confidence interval; *HiSCR*, hidradenitis suppurativa clinical response; *IHS4*, International Hidradenitis Suppurativa Severity Score; —, not applicable.

with a 39.79% reduction in IHS4 score in adjusted analysis (b = −39.79; 95% CI −54.92 to −24.65; $P < .001$). Each unit increase in BMI (as a continuous variable) attenuated the percentage change in IHS4 score by 1.65% (b = 1.65; 95% CI 0.50-2.81; $P = .01$). No other significant covariates were identified.

DISCUSSION

The effect of substituting HiSCR with IHS4 as the primary outcome measure of the PIONEER phase 3 clinical trials depended on whether the outcome variable was binary or continuous. Substituting HiSCR with change in IHS4 category resulted in no statistically significant difference between adalimumab and placebo in PIONEER 1 (Table II). Continuous variables (both nodule counts and IHS4 values) were statistically significant in both studies. Integrating draining tunnel status (using the IHS4) reduced placebo response rates in both PIONEER 1 and 2 regardless of whether a binary or continuous

Table IV. Linear regression model of change in nodules (model 1) and % change in International Hidradenitis Suppurativa Severity Score outcome measure (model 2) in adalimumab-treated patients in PIONEER 1 and PIONEER 2

	PIONEER 1			PIONEER 2		
Variable	**Estimate**	**95% CI**	***P* value**	**Estimate**	**95% CI**	***P* value**
Model 1			Change in nodules			
Adalimumab	**−2.36**	**(−4.40 to −0.31)**	**.02**	**−2.58**	**(−3.97 to −1.19)**	**<.001**
Hurley stage 3	**2.23**	**(−0.01 to 4.48)**	**.05**	−0.17	(−1.63 to 1.29)	.82
Family history	−0.74	(−3.19 to 1.72)	.56	−0.59	(−2.17 to 0.99)	.47
Current smoker	−1.00	(−3.08 to 1.08)	.35	−0.05	(−1.56 to 1.46)	.94
Presence of draining tunnels	1.09	(−1.49 to 3.66)	.41	**1.87**	**(0.32 to 3.43)**	**.02**
Antibiotic use	—	—	—	1.10	(−0.67 to 2.88)	.22
BMI	−0.04	(−0.18 to 0.09)	.54	−0.01	(−0.11 to 0.10)	.89
Male sex	−0.46	(−2.67 to 1.75)	.68	0.02	(−1.55 to 1.60)	.98
Age	0.03	(−0.06 to 0.12)	.51	0.03	(−0.03 to 0.09)	.38
Model 2			% Change in IHS4			
Adalimumab	**−18.60**	**(−33.64 to −3.55)**	**.02**	**−39.79**	**(−54.92 to −24.67)**	**<.001**
Hurley stage 3	8.45	(−6.89 to 23.80)	.28	2.54	(−12.83 to 17.90)	.75
Family history	−3.61	(−21.50 to 14.28)	.69	−6.59	(−23.77 to 10.59)	.45
Current smoker	1.29	(−14.04 to 16.63)	.87	4.68	(−11.71 to 21.07)	.57
Antibiotic use	—	—	—	16.75	(−2.52 to 36.02)	.09
BMI	0.17	(−0.82 to 1.15)	.74	**1.65**	**(0.50 to 2.81)**	**.01**
Male sex	10.61	(−5.52 to 26.75)	.20	8.82	(−7.70 to 25.34)	.29
Age	0.27	(−0.40 to 0.94)	.43	−0.04	(−0.72 to 0.65)	.92

BMI, Body mass index; *IHS4*, International Hidradenitis Suppurativa Severity Score.

variable was used. The use of the IHS4 as a continuous variable resulted in placebo response rates consistent with those of studies of psoriasis and atopic dermatitis (4.5%-12%).[10-13] This suggests that the placebo response rate is partially a product of the HiSCR outcome measure (ie, the use of a binary outcome), as well as the natural variability of inflammatory lesions in hidradenitis suppurativa and interrater variation in counting lesions.[3] It is recognized that the use of dichotomous outcomes reduces the power to detect difference between groups, increases the risks of false-positive results, and subsumes the variability in response within a group or cohort.[14] Because the IHS4 score is weighted toward abscesses and draining tunnels as opposed to nodules, it can be hypothesized that tunnels are less susceptible to such variability compared with superficial nodules, and hence the resolution of drainage is more indicative of successful therapy. The interrater variability of counting nodules has been previously identified as a factor contributing to placebo response rates,[3] and recent proposals for outcome measures assessing disease severity that do not include counts may provide a novel approach once validated in larger cohorts.[15] The association of elevated placebo response rates with specific outcome measures is an important finding, given the recent nonsignificant results of clinical trials evaluating C5a antagonists in hidradenitis suppurativa.[3,10] A recent phase 2b trial concluded that IFX-1 (InflaRx, Jena, Germany) was nonsuperior to placebo as measured by the HiSCR, with a placebo response rate of 47.2%. Post hoc analysis identified a significant reduction in draining tunnels compared with placebo as well as quality-of-life outcomes.[10]

Severe disease (assessed by the presence of draining tunnels) was significantly associated with a reduction in achieving HiSCR in PIONEER 2 (Table III), and Hurley stage 3 disease was associated with reduced odds of achieving IHS4 category change (Table III). In linear regression modeling, Hurley stage 3 disease was associated with an altered mean change in nodule count in PIONEER 1, and draining tunnels were associated with an altered mean change in nodule count in PIONEER 2. These results are consistent with the fact that severe disease (manifested either in increased Hurley staging or presence of draining tunnels) is less responsive to adalimumab therapy. BMI was significant in reducing HiSCR achievement, IHS4 category improvement, and percentage change in IHS4 in PIONEER 2. Every unit increase in BMI decreased the OR of achieving HiSCR by 7.1%, IHS4 category improvement by 4.9%, and the percentage change in IHS4 by 1.57%. The possibility of weight-based responses to current dosages of adalimumab in hidradenitis suppurativa requires further

investigation. Smoking is known to affect the efficacy of adalimumab in Crohn's disease,[16] and this is mirrored in hidradenitis suppurativa with our results (Table III, PIONEER 2 HiSCR).

The presence of any draining tunnels was significantly associated with HiSCR in PIONEER 2 but was not significantly different between adalimumab and placebo groups (Table I). Therefore, we conclude that draining tunnels is not a confounder on the effect of adalimumab in hidradenitis suppurativa but does have a significant association with HiSCR and change in nodule counts. The discrepancies in results between PIONEER 1 and 2 studies may be attributable to statistically significant differences in baseline patient characteristics (Supplemental Table I; available at https://doi.org/10.17632/7jmyytrzyx.1. Statistically significant differences were observed in race, age, BMI, smoking status, and presence of draining tunnel, and the median nodule and draining tunnel counts were significantly lower in PIONEER 2 compared with PIONEER 1. Additionally, baseline IHS4 scores were higher in PIONEER 1, indicating patients had more severe disease in PIONEER 1 than PIONEER 2.

The results of our analysis concur with those of Kimball et al[2] in that draining tunnels are not a confounder on the effect of adalimumab in hidradenitis suppurativa. However, our results go farther in identifying that draining tunnels, smoking status, antibiotic use, and BMI have an effect on clinical response as measured by HiSCR. These effects were more prominent in PIONEER 2 in the presence of less severe disease, suggesting they may influence response to adalimumab in patients with a disease severity similar to that of those included in PIONEER 2. Using an outcome measure that integrates draining tunnels (IHS4) identifies individuals with Hurley stage 3 disease as having reduced odds of achieving a change in IHS4 severity category compared with those with Hurley stage 2 disease. Stage 3 patients also exhibited a decreased change in nodule counts in the setting of adalimumab therapy compared with placebo. This suggests that despite the recent discussion in regard to the lack of biological correlation between Hurley staging and disease severity,[17] Hurley stage 3 disease has a statistically significant effect on the reduction of nodules in the setting of adalimumab therapy.

The limitations of this study include the inherent limitations of using clinical trial data, with the PIONEER studies not being an accurate representation of actual clinical practice. They also exclude the most severe cases of hidradenitis suppurativa, given that more than 20 draining tunnels was an exclusion criterion. Additionally, the data analyzed included only 12 weeks of therapy, but independent analysis suggests that response at week 12 is associated with clinical response at week 36 of therapy.[18]

The potential clinical applications of our findings are immediate in that treatment with adalimumab before the development of draining tunnels and Hurley stage 3 disease may be more efficacious. The statistically significant association with BMI also suggests that patients with increased BMI may have a decreased clinical response to adalimumab; however, it is unclear whether the degree of change (Tables III and IV) reaches clinical significance. Further investigation into the weight-based response to adalimumab in hidradenitis suppurativa is warranted, given these preliminary findings.

CONCLUSIONS

Adalimumab 40 mg weekly is effective in reducing clinical disease activity as measured by both the HiSCR and the IHS4 compared with placebo in participants with hidradenitis suppurativa. High placebo response rates may be a product of the use of binary outcome measures such as HiSCR. Regression analyses identified draining tunnels, smoking, antibiotic use, and BMI as independent significant associations with clinical response to adalimumab as measured by HiSCR in PIONEER 2. Only BMI was significantly associated with the use of percentage change in IHS4 in PIONEER 2. Future placebo-controlled studies of novel therapies in hidradenitis suppurativa should acknowledge the influence of outcome measure in the interpretation of their data.

REFERENCES

1. Kimball AB, Jemec GB, Yang M, Kalgeleriy A, Signorovith JE. Okun MM Assessing the validity, responsiveness and meaningfulness of the hidradenitis suppurativa clinical response (HiSCR) as the clinical endpoint for hidradenitis suppurativa treatment. *Br J Dermatol*. 2014;171(6):1434-1442.
2. Kimball AB, Sobell JM, Zouboulis CC, Gu Y, Williams DA, Sundaram M. HiSCR (hidradenitis suppurativa clinical response): a novel clinical endpoint to evaluate therapeutic outcomes in patients with hidradenitis suppurativa from the placebo controlled portion of a phase 2 adalimumab study. *J Eur Acad Dermatol Venereol*. 2016;30(6):989-994.
3. Ali AA, Seng EK, Alavi A, Lowes MA. Exploring changes in placebo treatment arms in hidradenitis suppurativa randomized clinical trials: a systematic review. *J Am Acad Dermatol*. 2020;82(1):45-53.
4. Frew JW. Assessing the efficacy of new biologic therapies in hidradenitis suppurativa: consistency vs bias in outcome measures in moderate and severe disease. *J Eur Acad Dermatol Venereol*. 2019;33(8):e298-e300.
5. Zouboulis CC, Tzellos T, Kyrgidis A, et al. Development and validation of the International Hidradenitis Suppurativa

Severity Scoring System (IHS4), a novel dynamic scoring system to assess HS severity. *Br J Dermatol*. 2017;177(5):1401-1409.
6. Micheletti RG Natural history, presentation and diagnosis of hidradenitis suppurativa. *Semin Cutan Med Surg*. 2014;33(3):S51-S53.
7. Kimball AB, Okun MM, Williams DA, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. *N Engl J Med*. 2016;375:422-434.
8. Zouboulis CC, Okun MM, Prens EP, Gniadecki R, Foley PA. Lynde C Long-term adalimumab efficacy in patients with moderate to severe hidradenitis suppurativa/acne inversa: 3-year results of a phase 3 open-label extension study. *J Am Acad Dermatol*. 2019;80(1):60-69.
9. R Core Team (2017). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Project for Statistical Computing.
10. InflaRX NV. InflaRx announces Top-Line SHINE phase IIb results for IFX-1 in hidradenitis suppurativa. Available at: https://www.inflarx.de/Home/Investors/Press-Releases/06-2019-InflaRx-Announces--Top-Line-SHINE-Phase-IIb-Results-for-IFX-1-in-Hidradenitis-Suppurativa-.html. Accessed January 24, 2020.
11. Reich K, Burden AD, Eaton JN, Hawkins NS. Efficacy of biologics in the treatment of 357 moderate to severe psoriasis: a network meta-analysis of randomized controlled trials. *Br J Dermatol*. 2012;166:179-188.
12. Oldhoff JM, Darsow U, Werfel T, et al. Anti-IL-5 recombinant humanized monoclonal 360 antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy*. 2005;60:693-696.
13. Simpson EL, Bieber T, Guttman-Yassky E, et al. Two phase 3 trials of dupilumab versus placebo in atopic dermatitis. *N Engl J Med*. 2016;375:2335-2348.
14. Altman DG, Royston P. *The cost of dichotomizing continuous variables BMJ*. 2006;332(7549):1080.
15. Kirby J, Butt M, King T. Severity and Area Score for Hidradenitis (SASH): a novel outcome measurement for hidradenitis suppurativa. *Br J Dermatol*. 2020;182(4):940-948.
16. Zorzi F, Zuzzi S, Onali S, Calabrese E. Efficacy and safety of infliximab and adalimumab in Crohn's disease: a single centre study. *Ailment Pharmacol Ther*. 2012;35(12):1397-1407.
17. Horvath B, Janse IC, Blok JL, et al. Hurley staging refined: a proposal by the Dutch Hidradenitis Suppurativa. *Expert Group Acta Derm Venereol*. 2017;97(3):412-413.
18. Jemec GBE, Okun MM, Forman SB, et al. Adalimumab medium-term dosing strategy in moderate-to-severe hidradenitis suppurativa: integrated results from the phase III randomized placebo-controlled PIONEER trials. *Br J Dermatol*. 2019;181(5):967-975.

4.5.3: Publication 5-3

Frew JW "Primary Imputation Methods Impact Efficacy Results in Hidradenitis Suppurativa Clinical Trials" J Am Acad Dermatol 2020; 83(2):663-665

5. Ghoufi L, Ortonne N, Ingen-Housz-Oro S, et al. Histiocytoid Sweet syndrome is more frequently associated with myelodysplastic syndromes than the classical neutrophilic variant: a comparative series of 62 patients. *Medicine (Baltimore)*. 2016; 95:e3033.

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Primary imputation methods impact efficacy results in hidradenitis suppurativa clinical trials

To the Editor: Missing data is a common issue in clinical trials. Analytic management of missing data involves including participants who drop out of a study in an intention-to-treat (ITT) analysis.[1] Analyzing only participants who complete a trial (per protocol [PP]) can eliminate missing data but at the expense of statistical power and external validity.[1] If the participant and disease-associated characteristics of those who completed the trial are representative of the ITT population, then PP can be valid. This is particularly important in placebo-controlled trials, where using PP may decrease the ability to detect a difference between groups (because only participants receiving placebo with a positive response tend to stay in the trial).[1]

In ITT, management of missing data involves primary imputation of missing values (Table I).[1] Multiple imputation involves complex statistical modeling and is beyond the scope of this discussion; the reader is directed to the statistical literature (Supplemental Materials; available via Mendeley at https://doi.org/10.17632/h8734gr7bc.1).[1] Primary imputation involves allocating participants a response based on the reasons/assumptions for missing data. Data can be classified as *missing not at random* (due to treatment-related factors, eg, lack of efficacy), *missing at random* (due to other documented factors such as age/sex that can be taken into account in multiple imputation), or *missing completely at random* (due to other undocumented variables not related to disease/treatment).[1] A sensitivity analysis (comparison of multiple primary imputation methods) is required to determine the effect of different analyses on the outcome(s) of interest.[1] This is especially pertinent given that clinical trial populations are not directly reflective of the general population.[1]

In the setting of hidradenitis suppurativa (HS), the high burden of disease and moderate therapeutic response rates may contribute to the high clinical trial dropout rates. The statistical methods used in these trials (Table II) vary, making comparisons complex. The PIONEER 1 and 2 studies were the only studies to conduct a sensitivity analysis,[2] conservatively presenting results of nonresponder imputation analysis. In contrast, the PIONEER Open Label Extension study[3] presented only PP data from a subset of participants, with last observation carried forward (LOCF) beyond week 96. This raises concerns regarding data validity, given that LOCF inflates response rates in long-term studies and is not recommended.[1] The use of ITT/nonresponder imputation in a randomized controlled trial of anakinra[4] in HS resulted in a loss of statistical significance. No dropout was seen in a phase 2a trial of IFX-1,[5] with differential attrition seen between arms of a phase 2 trial of bermekimab.[6] This may explain the apparent contradictory findings of an increased response rate in participants for whom anti–tumor necrosis factor (TNF) therapy failed (63%) when compared with anti-TNF–naive participants[6] (61%). Given that all participants who dropped out in the anti-TNF–failed arm achieved hidradenitis suppurativa clinical response, this may erroneously conflate the true efficacy of the drug in this population. In a cohort study of secukinumab,[7] 71% of the participants who dropped out did not achieve HiSCR, suggesting that LOCF presents a more conservative estimate of response compared with PP analysis, although the characteristics of the participants who

Table I. Definitions of primary imputation terms

Primary imputation term	Description
Missing equals success (MES)	Individuals with missing data are presumed to have achieved the endpoint of interest
Missing equals failure (MEF)	Individuals with missing data are presumed to have not achieved the endpoint of interest (equivalent to NRI)
Nonresponder imputation (NRI)	Individuals with missing data are presumed to have not achieved the endpoint of interest (equivalent to MEF)
Last observation carried forward (LOCF)	Individuals with missing data are presumed to have maintained the last observation, extrapolated forward to all future timepoints including endpoints of interest

Table II. Sensitivity analysis of missing data analysis in hidradenitis suppurativa clinical trials

Clinical trial	Reported primary outcome (method)	PP analysis, n/N (%)	ITT analysis, n/N (%)			Participants who dropped out, n
			MES	MEF (NRI)*	LOCF	
PIONEER 1 (adalimumab vs placebo)	Week 12 HiSCR INT: 41.8% (ITT/NRI)	64/145 (44.1)	72/153 (47.1)	64/153 (41.8)	—	8 outcome (s) unknown
	Week 12 HiSCR PBO: 26% (ITT/NRI)	40/145 (27.6)	49/154 (31.8)	40/154 (26)		9 outcome(s) unknown
		CMH: $P < .01$	CMH: $P < .01$	CMH: $P < .01$		
PIONEER 2 (adalimumab vs placebo)	Week 12 HiSCR INT: 58.9% (ITT/NRI)	96/155 (61.9)	104/163 (63.8)	96/163 (58.9)	—	8 outcome(s) unknown
	Week 12 HiSCR PBO: 27.6% (ITT/NRI)	45/151 (29.8)	57/163 (35.0)	45/163 (27.6)		12 outcome(s) unknown
		CMH: $P < .001$	CMH: $P < .001$	CMH: $P < .001$		
PIONEER OLE (adalimumab)	Week 12 HiSCR INT: (PP)†	46/88 (52.3)	—	—	Used for data >week 96	Unable to calculate
Anakinra (vs placebo)	Week 12 HiSCR INT: 7/9 (78%) vs PBO: 3/10 (30%) (PP)	INT: 7/9 (78)	INT: 8/10 (80)	INT: 7/10 (70)	INT: 7/10 (70)	1 outcome unknown
		PBO: 3/10 (20) χ^2: $P = .04$	PBO: 3/10 (30) χ^2: $P = .02$	PBO: 3/10 (30) χ^2: $P = .07$	PBO: 3/10 (30) χ^2: $P = .07$	0
IFX-1 (C5a)	Day 50 HiSCR: 9/12 (75%)	9/12 (75)	9/12 (75)	9/12 (75)	9/12 (75)	No dropout
Bermekimab (IL-1a)	Week 12 HiSCR: TNF failed (63%) (ITT LOCF)	13/22 (59)	17/24 (71)	13/24 (54)	15/24 (63)	2/2 achieved HiSCR (100%)
	Week 12 HiSCR TNF naive (61%) (ITT LOCF)	7/11 (64)	13/18 (72)	7/18 (39)	11/18 (61)	4/7 achieved (57%)
Secukinumab (IL-17A)	Week 12 HiSCR: 13/20 (65%) (ITT LOCF)	11/13 (85)	11/13 (85)	11/20 (55)	13/20 (65)	2/7 achieved HiSCR (29%)

CMH, Cochran-Mantel-Haenszel method; *HiSCR*, hidradenitis suppurativa clinical response; *IL*, interleukin; *INT*, intervention; *ITT*, intention to treat; *LOCF*, last observation carried forward; *MEF*, missing equals failure; *MES*, missing equals success; *NRI*, nonresponder imputation; *OLE*, Open Label Extension; *PBO*, placebo; *PP*, per protocol.

*MEF and NRI in the context of these studies are interchangeable.

†LOCF was imputed for timepoints beyond 96 weeks, as per the methods.

dropped out requires comparison to the overall cohort to assess the relationship between dropout and disease-related factors.

The decision about the best method of analysis is based on the individual study and the characteristics of the participants who dropped out. Future HS clinical trials should report and discuss participant dropout, and readers should critically evaluate the methods used to understand and acknowledge the potential bias in results.

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REFERENCES

1. Newgard CD, Lewis RJ. Missing data how to best account for what is not known. In: Livingston EH, Lewis RJ, eds. *JAMA Guide to Statistics and Methods*. New York, NY: McGraw-Hill; 2015.
2. Kimball AB, Okun MM, Williams DA, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. *N Engl J Med*. 2016; 375(5):422-434.
3. Zouboulis CC, Okun MM, Prens EP, Gniadecki R, Foley P. Lynde C Long-term adalimumab efficacy in patients with moderate-to-severe hidradenitis suppurativa/acne inversa: 3-year results of a phase 3 open label extension study. *J Am Acad Dermatol*. 2019;80(1):60-69.
4. Tzanatakou V, Kanni T, Giatrakou S, Katoulis A, Papadavid E, Netea MG. Safety and efficacy of anakinra in severe hidradenitis suppurativa: a randomized clinical trial. *JAMA Dermatol*. 2016;152(1):52-59.
5. Giamarellos-Bouboulis EJ, Argyropoulou M, Kanni T, et al. Clinical efficacy of complement C5a inhibition by IFX-1 in hidradenitis suppurativa: an open-label single arm trial for patients not eligible for adalimumab. [Epub ahead of print]. *Br J Dermatol*. 2020. https://doi.org/10.1111/bjd.18877. Accessed April 29, 2020.
6. Gottlieb A, Natsis NE, Kerdel F, et al. A phase II, open label study of bermekimab in patients with hidradenitis suppurativa shows resolution of inflammatory lesions and pain. [Epub ahead of print]. *J Invest Dermatol*. 2020. https://doi.org/10.1016/j.jid.2019.10.024. Accessed April 29, 2020.
7. Casseres RG, Prussick L, Zancanaro P, et al. Secukinumab in the treatment of moderate-to-severe hidradenitis suppurativa: results of an open-label trial. *J Am Acad Dermatol*. 2020; 82(6):1524-1526.

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Efficacy of oral zinc and nicotinamide as maintenance therapy for mild/moderate hidradenitis suppurativa: A controlled retrospective clinical study

To the Editor: Hidradenitis suppurativa (HS) is a chronic cutaneous disease that involves follicular occlusion in the apocrine gland-bearing regions. Treatment is a challenge because of the paucity of effective therapies and frequent exacerbations, with a negative impact on quality of life.[1] Brocard et al[2] first described zinc gluconate (90 mg daily for 4 months) as an effective therapeutic alternative for the management of HS. In our study, the efficacy of oral zinc and nicotinamide as maintenance treatment in mild to moderate HS was investigated retrospectively. A total of 92 patients affected by Hurley stage I and II HS were evaluated (Table I). All included patients had previously been treated with oral tetracycline (minocycline 100 mg daily) for 12 weeks with clinical and ultrasonographic benefit. The patients were divided into 2 groups according to treatment received or not received at the end of systemic antibiotic course. Specifically, 47 patients started oral therapy with capsules containing 90 mg of zinc gluconate and 30 mg of nicotinamide once daily for 90 days. The treated group was compared with a control group of 45 patients who did not receive any treatment. Each participant was evaluated at baseline and at 90 and 180 days after treatment. At 12 and 24 weeks, we observed a significant reduction in the number and mean duration of acute flares in the treated versus control groups. Patients of the treated group correspondingly reported a marked reduction in mean Visual Analogue Scale, Dermatology Life Quality Index, and International HS Severity Score System scores compared with the control group both at 12 and 24 weeks ($P < .005$). Disease-free survival was significantly longer in the treated group, and it showed sustained improvement even after discontinuation of oral supplementation. Slightly decreased or stable International HS Severity Score System score and pain Visual Analogue Score during the maintenance treatment was collaterally observed in the treated group with no statistically significant difference at 24 weeks (Table II). Two patients reported nausea; neither stopped the treatment. The use of oral zinc as a helpful treatment in HS (as monotherapy or in association with topical therapy) has been rarely described in the literature.[1-4] However, to our knowledge, no studies have investigated its usefulness as a maintenance treatment to potentiate the beneficial effects obtained with other agents, such as antibiotics, that are frequently used in HS. The efficacy of zinc could be related to its anti-inflammatory activity, inhibiting the chemotaxis of neutrophils, activating natural killer cells and the phagocytic function of granulocytes, and modulating the production of tumor necrosis factor α, interleukin 6, and metalloproteinases.[2,4] Additionally, it seems to have an antiandrogen activity, modulating 5α-reductase type I and II expression levels and activity.[2] Nicotinamide, as zinc, has anti-inflammatory and antioxidant activity by inducing the expression of the enzyme zinc/copper superoxide dismutase and reducing the accumulation of free radicals.[2,5] The main limitations of the study are the retrospective nature, with absence of a randomized, blinded control group. This study seems to suggest that zinc and nicotinamide supplementation in

4.5.4: Publication 5-4

Frew JW, Jiang CS, Singh N, Navrazhina K, Vaughan R, Krueger JG Quantifying the Natural Variation in Lesion Counts over time in Untreated Hidradenitis Suppurativa: Implications for Outcome Measures and Trial Design. JAAD International 2020; 1(2):208-221

Quantifying the natural variation in lesion counts over time in untreated hidradenitis suppurativa: Implications for outcome measures and trial design

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Background: Hidradenitis suppurativa (HS) demonstrates high placebo response rates in clinical trials, possibly due to the natural variability of the disease. No quantification of variability in lesion counts of untreated disease has been undertaken.

Objective: To quantify the variability of untreated HS.

Methods: Deidentified individual patient data from the placebo arms of PIONEER studies were analyzed, and measurements of within-subject coefficients of variation were examined. Variability was stratified by disease-associated variables (Hurley stage, BMI, sex, smoking, family history) and body site.

Results: Analysis of within-subject coefficients of variation demonstrated that half of the participants had a middle spread [difference between 75th and 25th percentiles of the subject's abscess and nodule counts] greater than 33% and 40% of their median abscess and nodule counts, and 25% of the subjects had a middle spread greater than 70% and 78% of their median abscess and nodule counts in PIONEER I and II, respectively. Hurley stage 2 participants had significantly greater within-subject variation than Hurley stage 3 patients. Variation was greater in the axillary and groin regions than in other anatomical locations.

Limitations: Limitations include the use of precollected clinical trial data.

Conclusion: The within-subject variability of the lesion counts in untreated HS was greater than previously appreciated. This has profound effects on outcome measures and the conduct of future clinical trials of HS. (JAAD Int 2020;1:208-21.)

Key words: acne inversa; clinical trials; hidradenitis suppurativa; natural history; variability.

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BACKGROUND

Hidradenitis suppurativa (HS) is a chronic inflammatory disease manifesting as painful nodules and abscesses and chronically draining tunnels in flexural areas.[1] Current outcome measures to assess disease severity in the clinic and in clinical trials rely upon counting the number of nodules, abscesses, and tunnels (also known as sinus tracts or fistulas).[2-4] The current gold standard outcome measure, HS clinical response (HiSCR),[5,6] is defined as a 50% reduction in the number of abscesses and nodules (AN count), with no increase in the number of abscesses or draining tunnels.[5,6] One known disadvantage of HiSCR is the elevated placebo response rates that occur with its use.[4,7] Placebo response rates in pivotal PIONEER studies of adalimumab for HS ranged between 26% and 27.6%, as measured by the percentage of participants achieving HiSCR.[7] Additionally, the placebo response rate in a phase 2b study of IFX-1 (a complement C5a) inhibitor, was very high at 47%,[4,8] with no statistically significant difference between the rates of HiSCR between the intervention and placebo arms.[4,8]

Factors that may contribute to the high placebo response rates include the sliding dichotomous nature of the outcome measure as well as the low inter-rater reliability of counting lesions.[2-4,9] A third aspect requiring further examination is the natural variation in lesion counts in untreated HS,[9] which remains incompletely defined.[4,9] Patient-reported retrospective questionnaires have been used to quantify the length of time for which the lesions persist (with 6.9 days reported for nodules).[10] However, given that clinical trials are based on an objective physician's evaluation of the clinical lesions,[7] the quantification of lesion variability needs to be assessed based on the physician's evaluation to maintain validity in the setting of clinical trials. A high level of test-retest reliability of the HiSCR outcome measure (R = 0.91) is at odds with the elevated placebo response rates previously described.[5] However, the primary endpoint for clinical trials is often at week 12 or 16,[7,11] suggesting that short-term observations may overestimate the stability of the lesion counts.

CAPSULE SUMMARY

- The natural variability of clinical disease in hidradenitis suppurativa remains incompletely defined.
- The within-subject variability of abscess and nodule counts in untreated hidradenitis suppurativa significantly contributes to placebo response rates and is greater for Hurley stage 2, axillary, and groin disease.

It has been acknowledged that individuals with AN counts of <3 at baseline should not be included in clinical trials as they would only require a one-lesion reduction (which may be in the range of natural disease fluctuation) in order to achieve HiSCR.[7] Additionally, such patients would not meet the criteria for moderate or severe disease. A number of studies have reported minimum AN counts of >5 in order to minimize issues with elevated placebo response rates[11]; however, the benefits are unclear given that the quantification of natural (untreated) disease variability has not been performed.[12]

The placebo arms of PIONEER I and II provided objective, physician-evaluated data for a period of 12 weeks of untreated (with the exception of topical antiseptic washes) moderate-to-severe HS. These data provide an opportunity to assess the natural variability of disease activity and develop a design for future clinical trials. Our overall aim was to assess the within-subject variability of lesion counts (inflammatory nodules, abscesses, draining tunnels, and AN counts) in participants enrolled in time period A (week 0 to week 12) in the placebo arms of the PIONEER I and PIONEER II phase 3 clinical trials using descriptive statistics and quantification of within-subject variations.

METHODS

Deidentified individual patient data from the PIONEER I and PIONEER II phase 3 studies of adalimumab therapy for HS were made available by AbbVie Inc and accessed through the secure Vivli online platform.[7] Raw data were extracted and compared with the available published data to ensure accuracy.[7] Data for participants allocated to the placebo arms in time period A (week 0 to week 12) were included in the analysis. Subjects were excluded if they had received any rescue antibiotics in PIONEER I or baseline concomitant antibiotics in PIONEER II. All data analyses were conducted using R version 3.5.3.[13]

Quantification of inflammatory nodules, abscesses, and draining tunnels (as previously defined) each available time point (Weeks 0, 2, 4, 6, 8, and 12) was undertaken.[5-7] As the lesion counts had skewed distributions (Fig 1), we examined 2 robust measures

Abbreviations used:

AN:	abscess and nodule
CVm:	median absolute deviation divided by the median
CVq:	interquartile range divided by the median
HiSCR:	hidradenitis suppurativa clinical response
HS:	hidradenitis suppurativa
IQR:	interquartile range
MAD:	median absolute deviation

of the within-subject coefficients of variation: (1) interquartile range (IQR) divided by the median (CVq) and (2) median absolute deviation (MAD) divided by the median (CVm). IQR is the difference between the 75th (Q3) and 25th percentile (Q1), the range of the middle half of the subject's data. Another measure of spread or dispersion that is more robust against outliers than IQR is (MAD). MAD is defined as the median of the absolute deviations from the subject's median value: MAD = median ($|x_i - \text{median}(x_i)|$), where x_i represents the repeated measurements for a subject. Both measures of within-subject variation (CVq and CVm) were multiplied by 100 to obtain a percentage.

To illustrate this with an example, consider a subject with AN counts of 11, 12, 9, 8, and 7 from baseline to week 12. The median value of this subject's data is 9, with Q1 = 8 and Q3 = 11 (IQR = 3). The absolute deviations about the median are 2, 3, 0, 1, and 2, which in turn have a median value of 2. MAD for these data is 2, and the within-subject CVm is 22% (2/9 × 100). The midpoint of the absolute differences about the subject's median is 22% of their median AN count. The within-subject CVq is 33% (3/9 × 100) and is interpreted as follows: the spread of the middle half of the subject's data is 33% of their median AN count. Higher percentages of the within-subject coefficient of variation indicate more dispersion around the median and higher within-subject variability over the 12-week period. Note that the MAD is 0 when majority of the subject's lesion counts are the same. For example, a subject with abscess counts of 1, 1, 1, 4, and 5 has a MAD of 0 and CVm of 0% (0/1 × 100), whereas the CVq is calculated as 300% (3/1 × 100).

Within-subject variation was also visualized using spaghetti plots and heat maps. Stratification by the type of lesions (inflammatory nodule, abscess, draining tunnel, AN count), known *a priori* treatment efficacy-associated factors (Hurley stage, gender, BMI category, nicotine use, family history), and anatomical location was undertaken. The Mann–Whitney U test was used to compare the AN count's CVq and CVm values by Hurley stage, gender, nicotine use, and family history. The Kruskal–Wallis test was used to compare the AN count's CVq and CVm values by BMI category (underweight/normal [<25 kg/m^2], overweight [25 to <30 kg/m^2], and obese [≥30 kg/m^2], according to the Centers for Disease Control and World Health Organization guidelines for adults). $P < .05$ was considered statistically significant.

RESULTS

Of the 633 available participants identified in PIONEER I and II, 262 participants in the placebo arms were included in the analysis (n = 141 in PIONEER I and n = 121 in PIONEER II). The baseline demographic and disease characteristics of the included subjects are presented in Table I. Baseline disease activity was more severe in the PIONEER I subjects, with higher median lesion counts, IHS4 score, and proportion of subjects with Hurley stage 3, than in the PIONEER II subjects. The distributions of the baseline lesion counts for both the PIONEER studies are presented in Fig 1. The heat maps and spaghetti plots of the lesion counts of the inflammatory nodules, abscesses, draining fistulas, and AN counts over time for PIONEER II are presented in Figs 2 and 3. Additional plots for PIONEER I are presented in Figs 4 to 6.

The calculated measurements of the within-subject variance, stratified by inflammatory nodules, abscesses, draining tunnels, and AN counts, are presented in Table II. The distributions of these measures were non-normal (Fig 7). The median within-subject CVq ranged from 33% to 50% for the lesion counts in both PIONEER I and II. The median CVq for the AN count was 33% (Q1 = 14%, Q3 = 70%) in PIONEER I and 40% (Q1 = 19%, Q3 = 78%) in PIONEER II. The interpretation is that half of the participants had middle spreads (difference between the 75th and 25th percentiles of the subject's AN counts) greater than 33% and 40% of their median AN count in the placebo arms of PIONEER I and II, respectively. The 75th percentile of CVq for the AN count suggested that 25% of the subjects had middle spreads greater than 70% and 78% of their median AN counts in PIONEER I and II, respectively.

The spaghetti plots of the within-subject absolute deviations from the medians for PIONEER I and II are presented in Fig 8. The median within-subject CVm for the lesion counts ranged from 4% to 17% for PIONEER I and from 11% to 25% for PIONEER II. The within-subject variation was lower for abscesses than

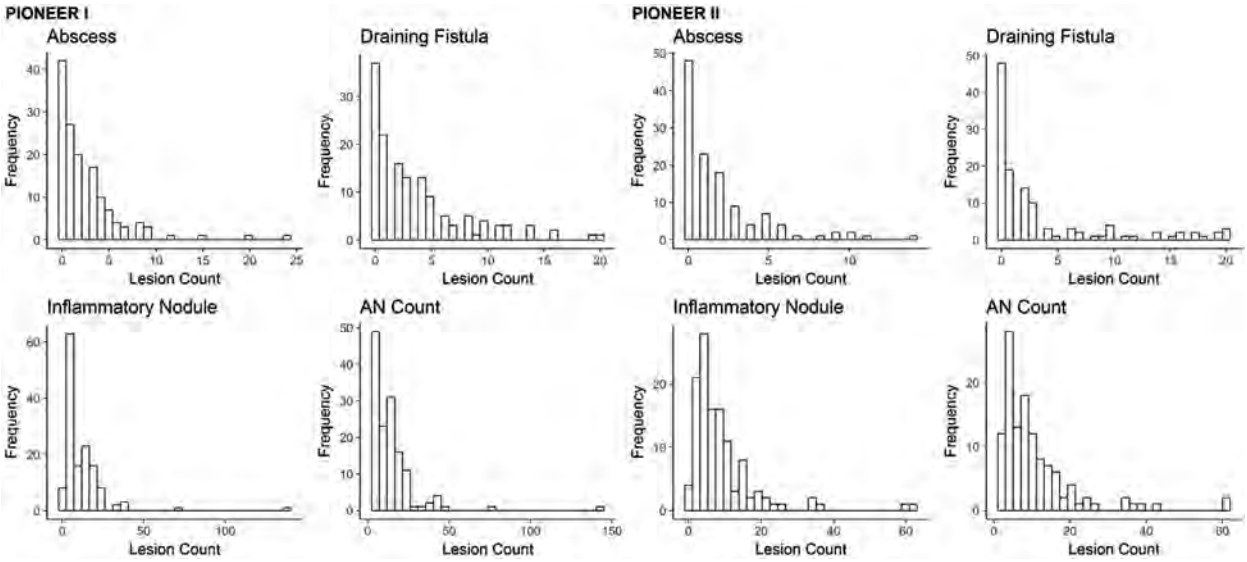

Fig 1. Histograms of the distribution of baseline disease activity in the PIONEER I and PIONEER II trials.

Table I. Baseline characteristics of the subjects included in the analysis

Characteristic	PIONEER I placebo (n = 141)	PIONEER II placebo (n = 121)
Gender		
Female	99 (70.2%)	90 (74.4%)
Male	42 (29.8%)	31 (25.6%)
Race		
White	110 (78.0%)	98 (81.0%)
Black	24 (17.0%)	15 (12.4%)
Other	7 (5.0%)	8 (6.6%)
Median Age*	36.0 (30.0-47.0)	33.0 (26.0-42.0)
Median BMI*	33.8 (28.4-39.3)	31.7 (26.5-36.5)
BMI category		
Underweight/normal	12 (8.5%)	22 (18.3%)
Overweight	35 (24.8%)	23 (19.2%)
Obese	94 (66.7%)	75 (62.5%)
Hurley stage		
2	75 (53.2%)	74 (61.2%)
3	66 (46.8%)	47 (38.8%)
Nicotine use	85 (60.3%)	89 (74.2)
Family history	28 (19.9%)	31 (25.6%)
Presence of draining tunnels	104 (73.8%)	73 (60.3%)
Median inflammatory nodules*	7 (4-15)	6 (4-10)
Median abscesses*	2 (0-3)	1 (0-3)
Median AN count*	11 (6-17)	8 (5-13)
Median draining tunnels*	2 (0-5)	1 (0-3)
Median baseline IHS4*	25 (13-40)	1 (9-30)

Data are presented as n values with percentages for categorical variables.
AN, Abscess and nodule.
*Data are presented as medians with interquartile ranges in parentheses.

for other lesion counts in PIONEER I when MAD was used as the measure of dispersion. Forty-three out of 87 subjects had the same abscess count for at least 3 out of the 5 visits, which resulted in a MAD of 0 and subsequently a CVm of 0%. The median CVm for the AN count was 16% (Q1 = 5%, Q3 = 31%) in PIONEER I and 20% (Q1 = 8%, Q3 = 39%) in PIONEER II. For half of the subjects, the MAD (middle of the absolute

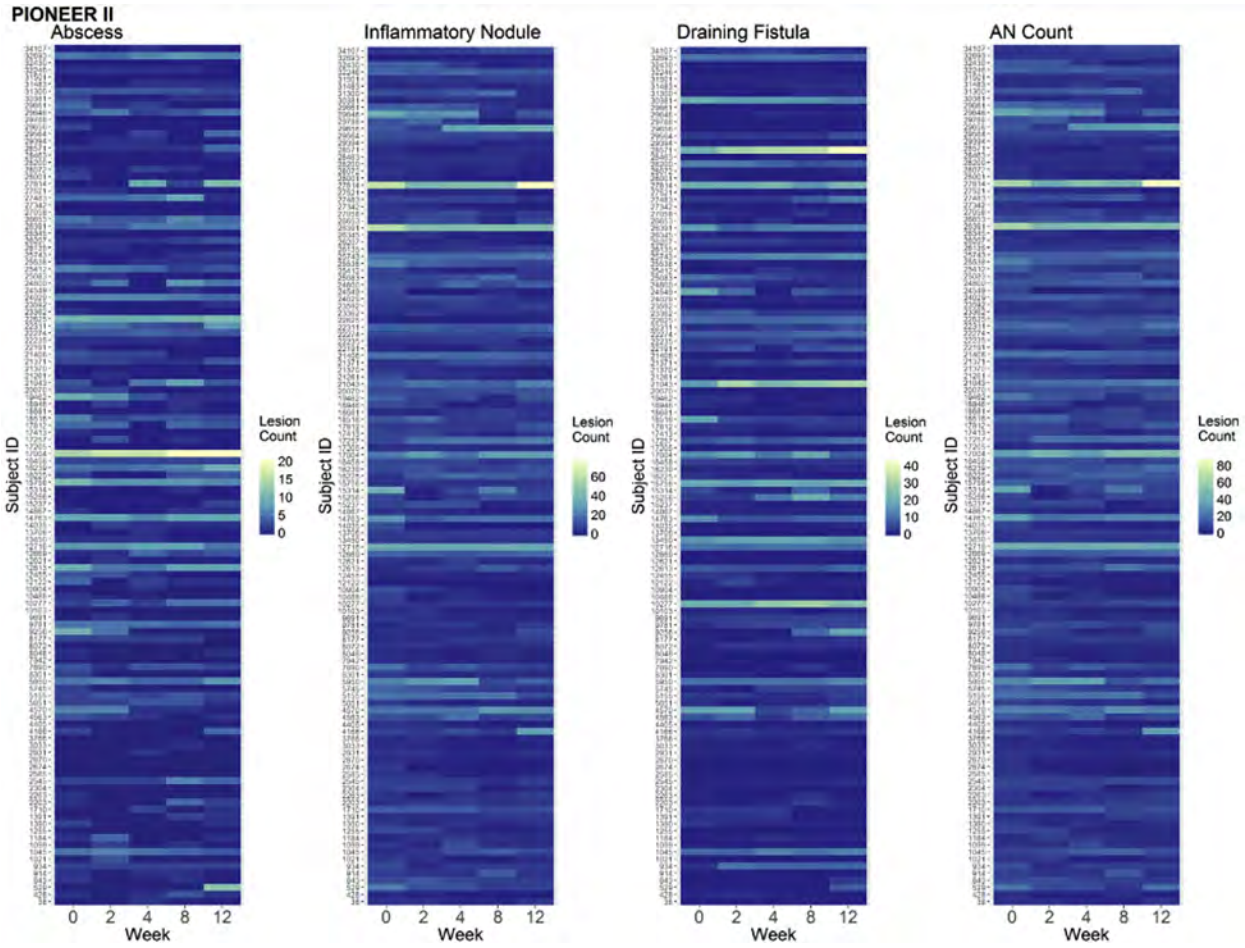

Fig 2. The heat maps of the lesion counts for the abscesses, inflammatory nodules, draining fistulas, and abscess and nodule (AN) counts during the 12-week time period of PIONEER II. These heat maps allow a visual representation of the within-subject variability for all the subjects included over time. The heat maps use a *blue-green-yellow* color scale, with the lowest count for each lesion type indicated in *blue* and the highest in *yellow*.

differences from the subject's median) was greater than 16% and 20% of their median AN counts in PIONEER I and II, respectively. For 25% of the subjects, the MAD was greater than 31% and 39% of their median AN counts in PIONEER I and II, respectively.

The small sample size for within-subject variation measures of the abscesses and draining tunnels in PIONEER I and II is noteworthy. The subjects in both the studies had low baseline counts for these lesions, and the coefficient of variation measures was undefined when divided by a median of 0. The within-subject variation measures of the AN count (sum of the number of abscesses and inflammatory nodule count important for determining the HiSCR response) were computable for 99.3% (140/141) of the subjects in PIONEER I and 97.5% (118/121) of the subjects in PIONEER II.

The stratification of the within-subject variation measures in AN counts by the *a priori* treatment efficacy-associated factors showed that the participants with Hurley stage 3 had significantly lower within-subject variability, as measured by CVq (Table III), compared to the participants with Hurley stage 2 in PIONEER I (P = .011) and II (P = .048). This difference was only observed in PIONEER I (P = .004) when CVm was used as the measure of within-subject variability (Table IV). No significant differences were observed when CVq and CVm were stratified by gender, BMI category, nicotine use, or family history.

The plots of the within-subject variations (measured by absolute deviations from the median AN counts) stratified by body site indicated that the axillary and inguinal regions demonstrated the greatest variability (Fig 9). The MAD was 0 across

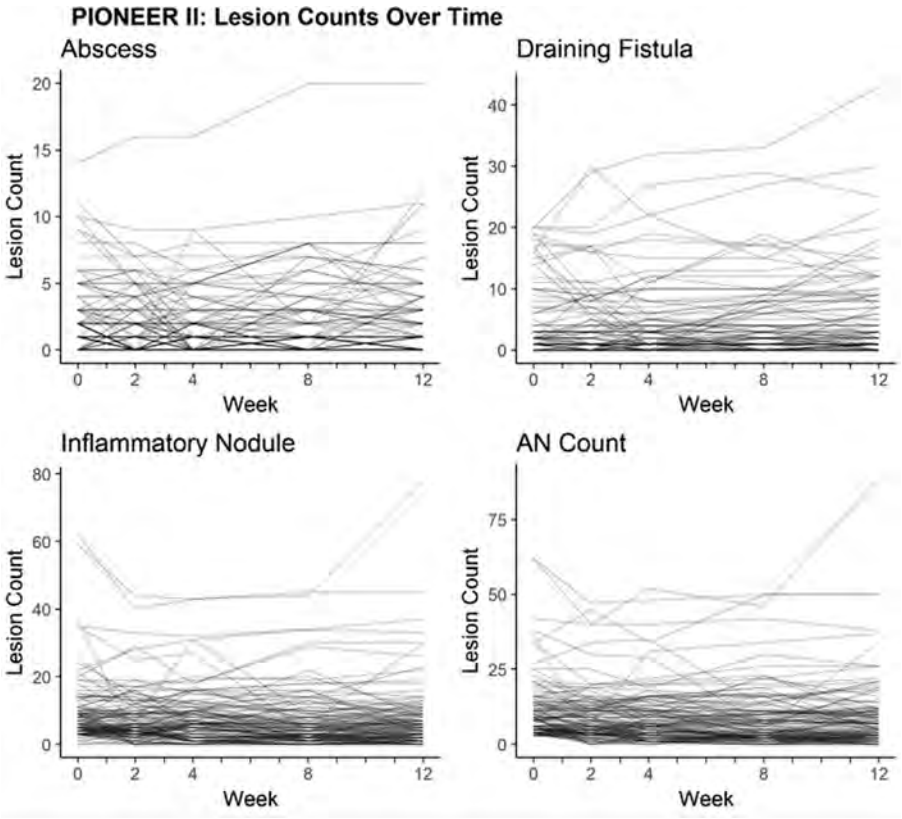

Fig 3. The spaghetti plots of the within-subject variability for abscesses, inflammatory nodules, draining fistulas, and abscess and nodule (AN) counts during the 12-week time period of PIONEER II. The x axis represents the absolute values of disease activity, and the y axis represents the time in weeks.

all body sites in PIONEER I and II. However, the means of their absolute deviations were consistently higher for the axillary and inguinal sites than for the other body sites (Table V). The measurements of CVq and CVm stratified by body site were not undertaken due to sparse AN counts when examining each body site separately. The CVq and CVm were calculated by dividing the median values by 0, which gave an undefined result across multiple body sites.

DISCUSSION

Our results have identified significant within-subject variability in the lesion counts in untreated participants from the placebo arms of the PIONEER I and II phase 3 studies. To our knowledge, this is the first report to quantify the natural variability of clinical HS and explain the high placebo response rates reported in the original studies (26.0% and 27.6%, respectively).[7] The median and IQR of the changes in the AN counts from the baseline (Table VI) and the placebo response rates were seen to progress over time (15.6% and 12.4% at week 2, respectively; 20.1% and 24.0% at week 4, respectively; 22.5% and 28.1% at week 8, respectively; and 27.7% and 30.6% at week 12, respectively) in both PIONEER I and II. This supports the concept of natural variation in the disease, accounting for the results presented.

Participants in PIONEER II had a higher within-subject variation in the AN count compared to those in PIONEER I based on the quartiles (40% vs 33%, respectively) and MAD (20% vs 16%, respectively), which supports the higher placebo response rates seen in PIONEER II at weeks 4, 8, and 12 compared to those seen in PIONEER I. In PIONEER I, the Hurley stage 3 subjects had significantly lower variance in the AN count when compared to those with Hurley stage 2 when both the measures of the within-subject coefficient of variation were used. This may be due to the difficulty in assessing the abscesses and inflammatory nodules in the presence of significant scarring. The higher placebo response rates in PIONEER II may be explained by the higher proportion of Hurley stage 2 subjects than those in PIONEER I (Table I). This implies that individuals

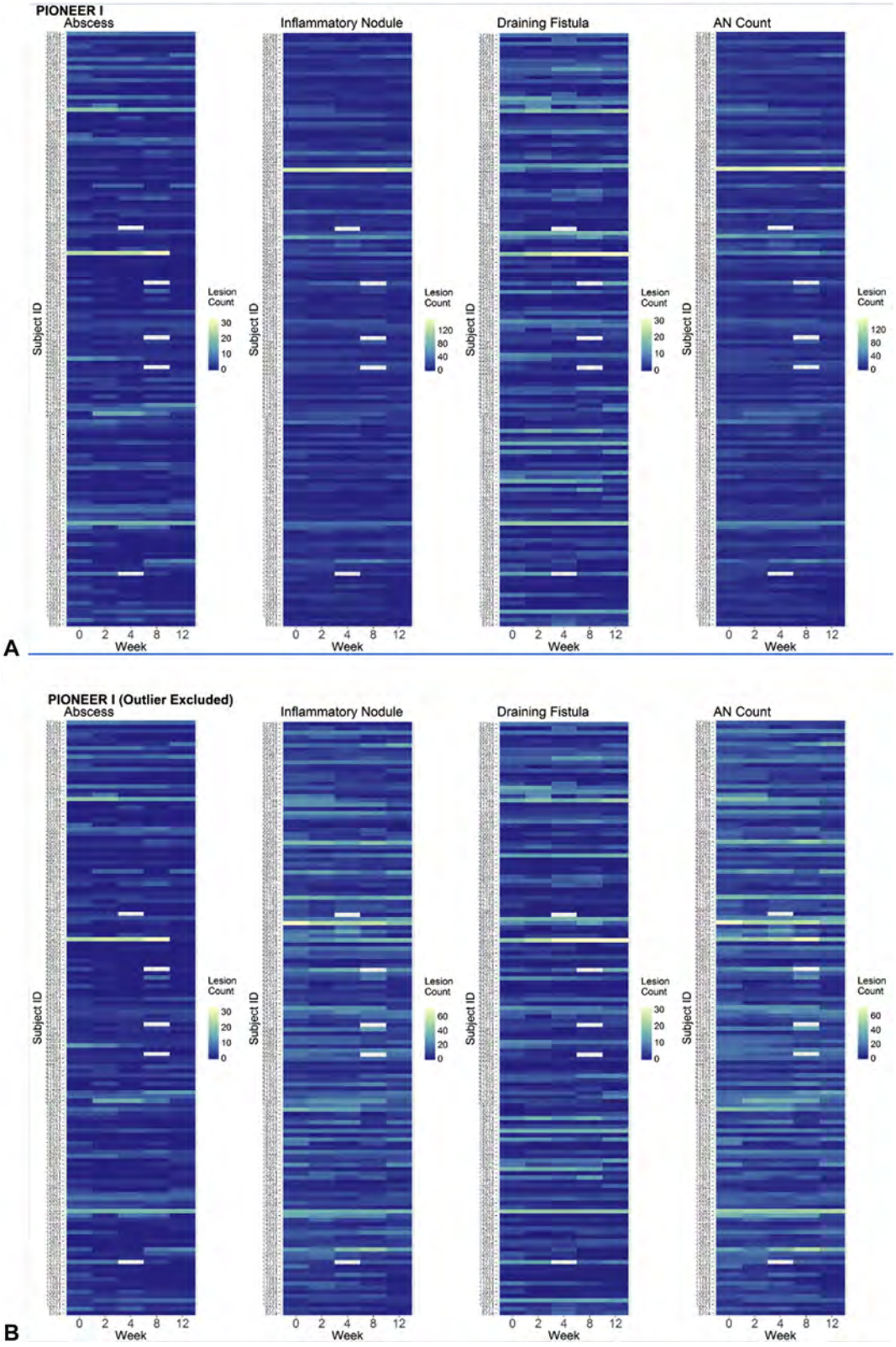

Fig 4. **A** and **B**, Heat maps of the lesion counts for the abscesses, inflammatory nodules, and draining fistulas and the abscess and nodule (AN) count during the 12-week time period of PIONEER I, with (A) and without (B) an outlier with consistently high inflammatory nodule counts throughout the 12-week period (this subject was included in all the analyses and excluded only here for visualization purposes).

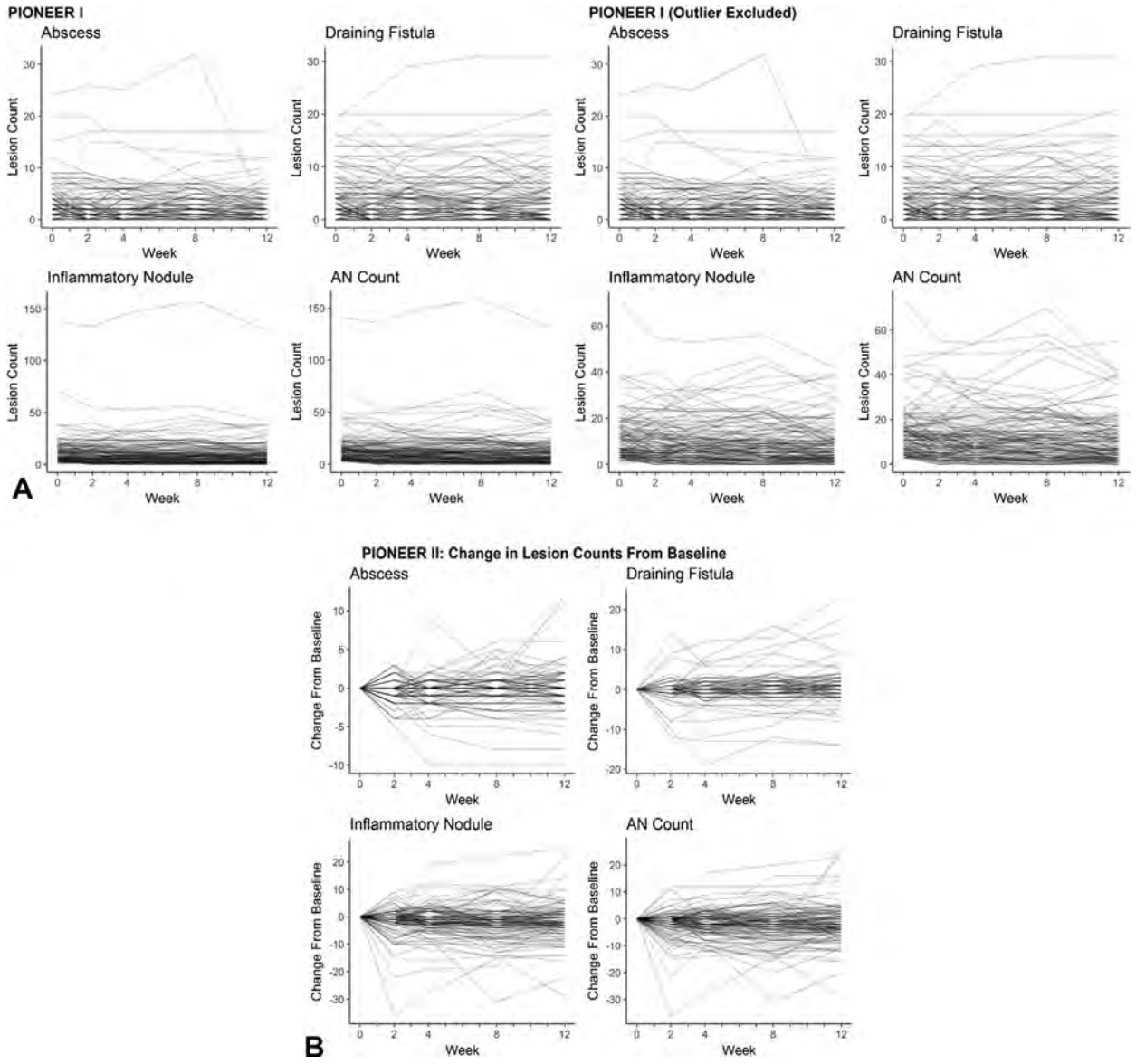

Fig 5. **A**, Spaghetti plots of the lesion counts for the abscesses, inflammatory nodules, draining fistulas, and abscess and nodule (AN) count during the 12-week time period of PIONEER I with and without an outlier with consistently high inflammatory nodule counts throughout the 12-week period (this subject was included in all analyses and excluded only for visualization purposes). **B**, Spaghetti plots of changes in the abscesses, inflammatory nodules, draining fistulas, and AN count during the 12-week time period of PIONEER II.

with lower disease severity are more prone to intra-subject variability.

Significant differences were identified when CVq was stratified by the morphological type of the lesion, with subepidermal lesions (abscesses/tunnels) having the greatest variation. Additionally, the within-subject variance was greater in the axillary and groin regions compared to that in the other anatomical locations. This variation between different anatomical sites may be inherent to the disease and of such a degree that it does not carry any clinical significance; however, it must be acknowledged that "counting fatigue" by investigators in clinical trials may also play a role.

We can confirm that the analyzed data included coding regarding which investigator performed which ratings. For all individuals, the same investigator performed the ratings at the baseline and at all the time points through week 12. This indicates that the variability is due to intrapatient variation and not

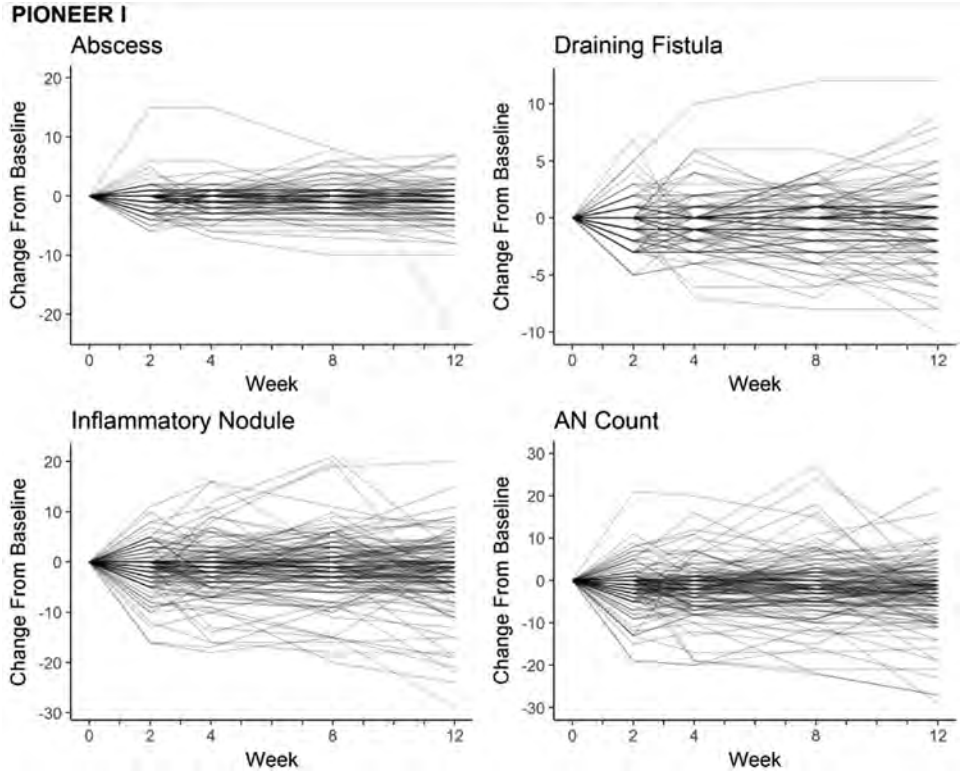

Fig 6. Spaghetti plots of change from baseline to each time point for abscess, inflammatory nodule, draining fistula, and AN count during period A (12 weeks) for PIONEER I.

Table II. The measures of within-subject variation in clinical disease activity of participants with hidradenitis suppurativa in the placebo arms of PIONEER I and II*

PIONEER I	Abscess (n = 87)	Inflammatory nodule (n = 137)	Draining fistula (n = 96)	AN count (n = 140)
Coefficient of variation quartiles (CVq) = IQR/ median × 100 (%)	50.0 (11.2, 100.0)	37.5 (18.8, 71.4)	40.0 (6.3, 100.0)	33.3 (14.0, 70.4)
Median absolute deviation (MAD) = median ($\lvert x_i - \text{median}(x_i)\rvert$)	0.0 (0.0, 1.0)	1.0 (0.0, 3.0)	0.0 (0.0, 1.0)	1.0 (1.0, 2.5)
Coefficient of variation MAD (CVm) = MAD/ median × 100 (%)	4.0 (0.0, 42.7)	16.7 (0.0, 37.5)	16.7 (0.0, 35.0)	15.8 (5.4, 31.4)
PIONEER II	**Abscess (n = 58)**	**Inflammatory nodule (n = 113)**	**Draining fistula (n = 73)**	**AN count (n = 118)**
Coefficient of variation quartiles (CVq) = IQR/ median × 100 (%)	45.0 (11.1, 100.0)	50.0 (25.0, 75.0)	33.3 (10.0, 100.0)	40.0 (18.7, 77.7)
Median absolute deviation (MAD) = median ($\lvert x_i - \text{median}(x_i)\rvert$)	0.0 (0.0, 1.0)	1.00 (1.00, 2.00)	0.0 (0.0, 1.0)	1.0 (1.0, 2.0)
Coefficient of variation MAD (CVm) = MAD/ median × 100 (%)	15.5 (0.0, 50.0)	25.0 (9.1, 44.4)	11.1 (0.0, 40.0)	20.0 (8.3, 39.4)

AN, Abscess and nodule; *IQR*, interquartile range (Q3-Q1); *MAD*, median absolute deviation; x_i, repeated measurements for a subject.
*Data are presented as medians with the 25th (Q1) and 75th (Q3) percentile values in parentheses. For the MAD calculations, n = 141 in PIONEER I and n = 121 in PIONEER II.

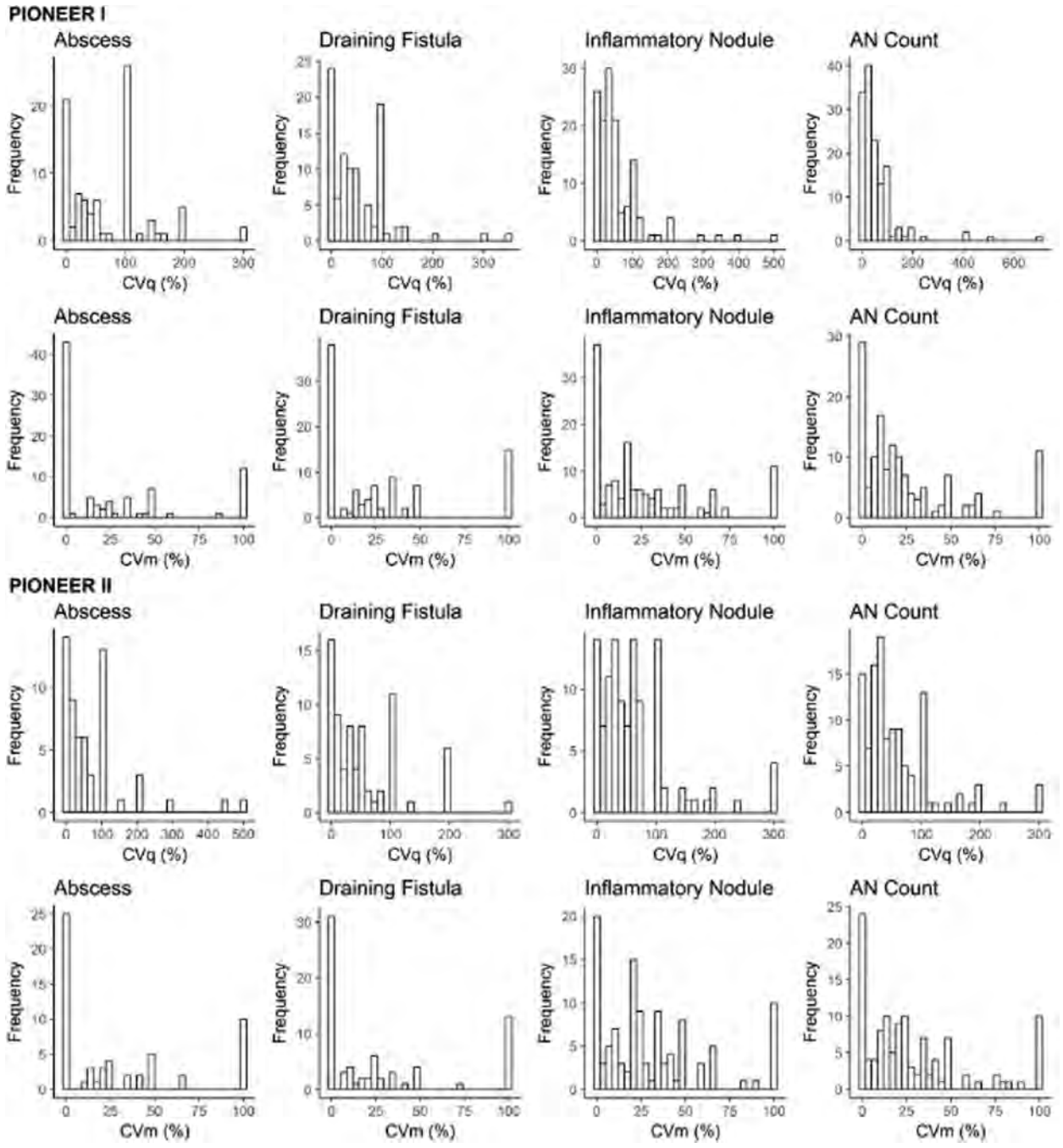

Fig 7. Histograms of the distribution of within-subject variability measures in PIONEER I and PIONEER II.

inter-rater reliability. The progression of the placebo response rates over time would be more consistent with the intrasubject variability because of the natural variation in the disease activity rather than with the undocumented switching of the investigators. If there was any undocumented switching of investigators, this has not been captured in the dataset examined.

This analysis is limited by the inherent aspects of using clinical trial data as well as the restrictions according to the inclusion and exclusion criteria of the studies of interest. Other large databases of real-life clinical data exist, such as UNITE registry[14]; however, all of those patients were exposed to some form of systemic therapy. The placebo arms of the PIONEER studies provide the largest cohort of

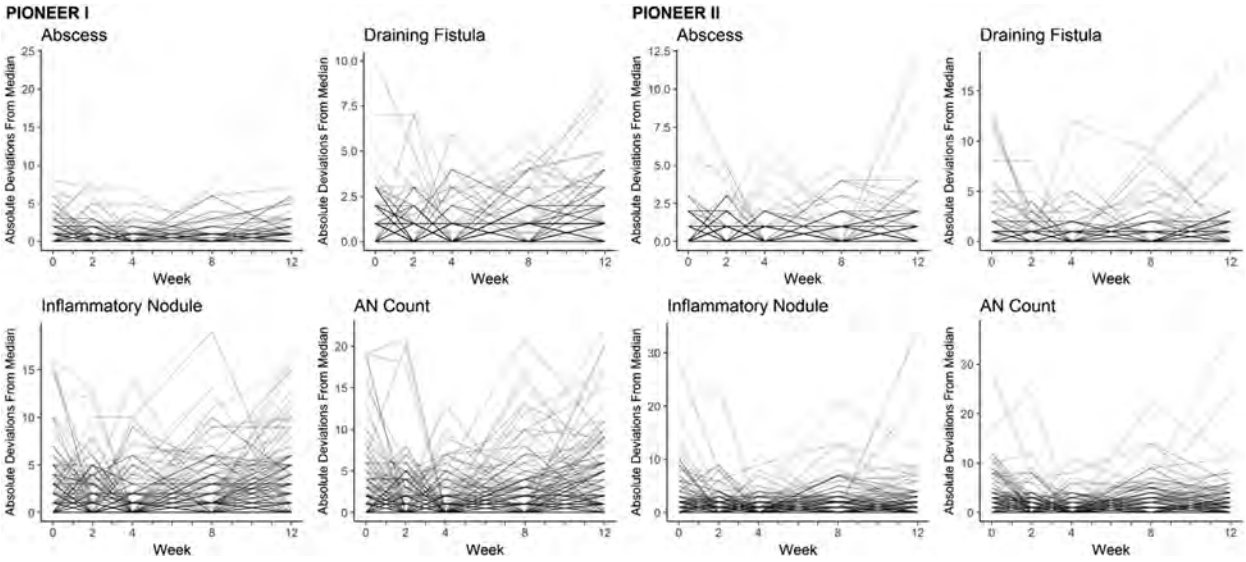

Fig 8. Spaghetti plots of the within-subject variability as measured by absolute deviations from the median for the abscesses, inflammatory nodules, draining fistulas, and abscess and nodule (AN) counts during the 12-week time period of PIONEER I and PIONEER II.

Table III. Within-subject variations in AN count as measured by coefficient of variation quartiles (cvq %) stratified by *a priori* features*

Characteristic	PIONEER I (n = 140)	*P* value	PIONEER II (n = 118)	*P* value
Gender				
Female	33.3 (14.9-72.0)	.684	40.0 (17.8-80.0)	.582
Male	32.1 (12.1-65.9)		33.3 (22.3-72.9)	
BMI category				
Underweight/normal	22.2 (13.6-55.7)		36.7 (17.8-100.0)	
Overweight	25.0 (11.1-71.3)	.607	64.5 (16.7-100.0)	.637
Obese	37.5 (16.7-70.0)		34.4 (21.8-66.7)	
Hurley stage				
2	48.5 (20.3-96.9)	**.011**	49.1 (26.0-100.0)	**.048**
3	23.8 (7.72-50.0)		33.3 (15.7-66.7)	
Nicotine use				
No	37.5 (14.6-66.2)	.939	34.4 (16.7-66.7)	.229
Yes	28.6 (13.2-80.0)		40.0 (20.0-83.7)	
Family history				
No	33.3 (14.8-72.5)	.518	35.9 (19.7-73.3)	.831
Yes	25.7 (11.1-58.8)		40.7 (16.7-96.9)	

AN, Abscess and nodule.
Bold numbers indicate statistical significance.
*Data are presented as medians with interquartile ranges in parentheses. Statistical analyses were conducted using Mann–Whitney U test for gender, Hurley stage, nicotine use, and family history and Kruskal–Wallis test for BMI category.

untreated patients for an extended time period, which is most applicable to investigate the placebo response rates in clinical trials. Further prospective studies of the natural histories of untreated patients are still needed and would further add to our knowledge in this aspect. The role of training investigators in improving the accuracy of HiSCR scoring and reducing variability remains unclear; however, the investigators in the PIONEER studies underwent HiSCR training,[7] suggesting that the high degree of intrasubject variation is a true product of clinical disease activity. Our analysis was also limited by the inability to assess the natural variation in disease activity from potential confounding factors such as "counting fatigue," undocumented use of multiple investigators at a single site, and variations

Table IV. Within-subject variation in the AN count as measured by the coefficient of variation (cvm) based on the median absolute deviation stratified by *a priori* features*

Characteristic	PIONEER I (n = 140)	*P* value	PIONEER II (n = 118)	*P* value
Gender				
Female	16.23 (7.69, 32.69)		22.22 (10.00, 40.00)	
Male	14.36 (3.95, 30.22)	.527	13.39 (0.00, 31.25)	.123
BMI category				
Underweight/normal	11.11 (0.00, 36.36)		18.33 (0.00, 91.67)	
Overweight	14.29 (6.97, 34.29)		19.35 (10.42, 50.00)	
Obese	16.67 (5.26, 30.77)	.856	20.00 (9.32, 33.33)	.801
Hurley stage				
2	22.22 (10.00, 48.86)		22.65 (10.39, 40.00)	
3	11.11 (2.46, 20,00)	**.004**	14.84 (4.25, 30.78)	.12
Nicotine use				
No	16.67 (5.85, 28.57)		18.01 (5.63, 33.33)	
Yes	15.38 (5.00, 33.33)	.988	20.00 (8.33, 40.00)	.605
Family history				
No	16.23 (5.41, 31.41)		20.00 (7.50, 38.12)	
Yes	13.45 (4.69, 31.41)	.824	22.50 (8.75, 39.38)	.684

Bold numbers indicate statistical significance.
*Data are presented as medians with the 25th and 75th percentiles (Q1, Q3). Statistical tests were conducted using the Mann–Whitney U test for gender, Hurley stage, nicotine use, and family history and the Kruskal–Wallis test for BMI category.

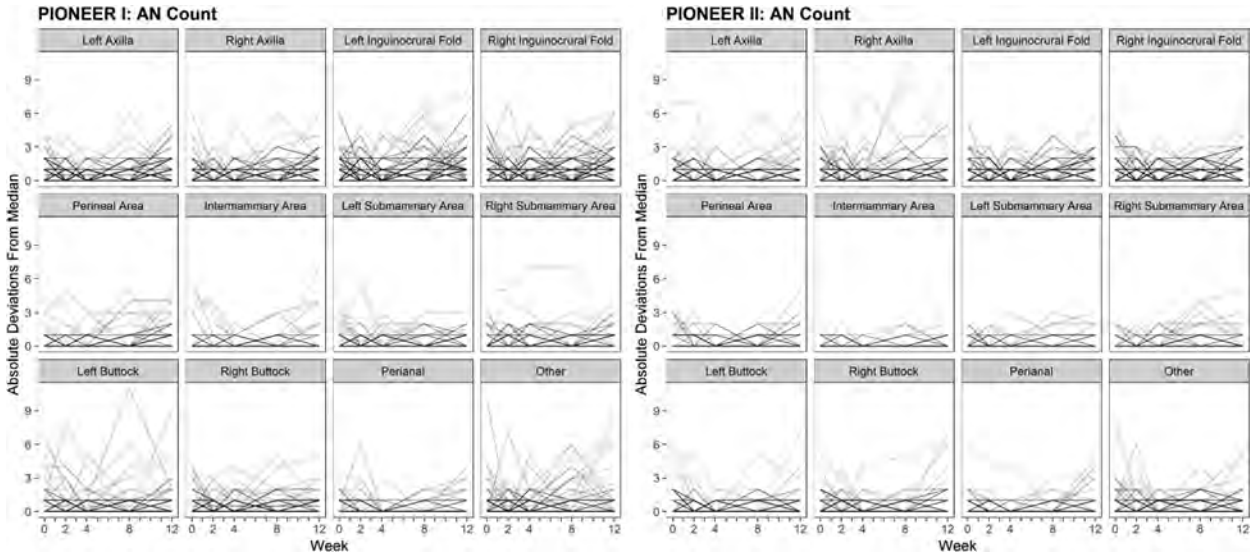

Fig 9. Spaghetti plots of the within-subject variability as measured by absolute deviations from the median for the abscess and nodule (AN) counts during the 12-week time period of PIONEER I and PIONEER II, stratified by body site.

in the counting methods between different investigators.

In order to address the identified within-subject variance and reduce the placebo response rates, various options could be employed in future HS clinical trials. Increasing the minimum number of lesions for inclusion in clinical trials would reduce the external validity of future trials and is not recommended. Elevating the clinical response definition from a 50% reduction to a >75% reduction (>third quartile of CVq) in the AN count (HiSCR-75) would reduce the placebo response rates. Analyzing PIONEER II data based on HiSCR-75 would result in a placebo response rate of 15%, and 36% of those in the adalimumab arm would achieve HiSCR-75 ($P < .0001$). The third option would be to develop and validate a new clinical outcome measure that does not involve counting lesions and sliding dichotomous outcomes.

Table V. Stratification of the within-subject MAD stratified by body site*

Body site	PIONEER 1 (n = 141)		PIONEER 2 (n = 121)	
	Mean (min, max)	Median (Q1, Q3)	Mean (min, max)	Median (Q1, Q3)
Axilla				
Left axilla	0.22 (0.00, 2.00)	0.00 (0.00,0.00)	0.21 (0.00, 2.00)	0.00 (0.00, 0.00)
Right axilla	0.23 (0.00, 2.00)	0.00 (0.00, 0.00)	0.29 (0.00, 2.00)	0.00 (0.00, 0.00)
Groin				
Left inguinocrural fold	0.41 (0.00, 2.00)	0.00 (0.00, 1.00)	0.37 (0.00, 2.00)	0.00 (0.00, 1.00)
Right inguinocrural fold	0.40 (0.00, 3.00)	0.00 (0.00, 1.00)	0.36 (0.00, 2.00)	0.00 (0.00, 1.00)
Perineal area	0.13 (0.00, 3.00)	0.00 (0.00, 0.00)	0.10 (0.00, 2.00)	0.00 (0.00, 0.00)
Submammary				
Intermammary area	0.05 (0.00, 2.00)	0.00 (0.00, 0.00)	0.03 (0.00, 1.00)	0.00 (0.00, 0.00)
Left submammary area	0.09 (0.00, 2.00)	0.00 (0.00, 0.00)	0.07 (0.00, 2.00)	0.00 (0.00, 0.00)
Right submammary area	0.14 (0.00, 5.00)	0.00 (0.00, 0.00)	0.05 (0.00, 2.00)	0.00 (0.00, 0.00)
Buttock				
Left buttock	0.19 (0.00, 4.00)	0.00 (0.00, 0.00)	0.14 (0.00, 3,00)	0.00 (0.00, 0.00)
Right buttock	0.17 (0.00, 4.00)	0.00 (0.00, 0.00)	0.13 (0.00, 4.00)	0.00 (0.00, 0.00)
Perianal	0.06 (0.00, 1.00)	0.00 (0.00, 0.00)	0.08 (0.00, 4.00)	0.00 (0.00, 0.00)
Other	0.21 (0.00, 3.00)	0.00 (0.00, 0.00)	0.17 (0.00, 2.00)	0.00 (0.00, 0.00)

MAD, Median absolute deviation.
*Data are presented as mean with ranges (min, max) or as medians with 25th and 75th percentiles (Q1, Q3).

Table VI. Changes in the AN count at each time point from the baseline*

Time point	PIONEER I (n = 141)	*P* value	PIONEER II (n = 121)	*P* value
Week 2	−1 (−3, 0)	.0002	0 (−3, 0)	<.0001
Week 4	−1 (−4, 1)	<.0001	−2 (−4, 1)	<.0001
Week 8	−2 (−5, 2)	.001	−2 (−6, 1)	<.0001
Week 12	−2 (−6, 0)	<.0001	−2 (−5, 1)	<.0001

AN, Abscess and nodule.
*Data are presented as medians with 25th and 75th percentiles (Q1, Q3). *P*-values were calculated using the Wilcoxon signed-rank test.

CONCLUSIONS

The natural variability in the lesion counts in the untreated participants in the placebo arms of PIONEER I and II was significantly larger than previously appreciated. This variation was greater among Hurley stage 2 than stage 3 subjects and also varied by body site. Clearly, if lesion counts continue to be used as primary outcomes, this higher than previously appreciated variability will have an untoward impact on the required sample sizes, costs, and time to completion for future clinical trials.

This publication is based on research data from AbbVie Inc, which have been made available through Vivli Inc. Vivli Inc and AbbVie Inc had no role in the design or execution of the study, statistical analysis, or composition of the manuscript.

REFERENCES

1. Sabat R, Jemec GBE, Matusiak L, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. *Nat Rev Dis Primers*. 2020;6(1):18.
2. Thorlacius L, Garg A, Riis PT, et al. Inter-rater agreement and reliability of outcome measurement instruments and staging systems used in hidradenitis suppurativa. *Br J Dermatol*. 2019; 181(3):483-491.
3. Kirby JS, Butt M, King T. Severity and Area Score for Hidradenitis (SASH): a novel outcome measurement for hidradenitis suppurativa. *Br J Dermatol*. 2020;182(4):940-948.
4. Frew JW, Jiang CS, Singh N, et al. Clinical response rates, placebo response rates, and significant associated covariates are dependent on choice of outcome measure in hidradenitis suppurativa. A post hoc analysis of PIONEER 1 and 2 individual patient data. *J Am Acad Dermatol*. 2020;82(5):1150-1157.
5. Kimball AB, Jemec GB, Yang M, Kalgeleriy A, Signorovith JE, Okun MM. Assessing the validity, responsiveness and meaningfulness of the Hidradenitis Suppurativa Clinical Response (HiSCR) as the clinical endpoint for hidradenitis suppurativa treatment. *Br J Dermatol*. 2014;171(6):1434-1442.
6. Kimball AB, Sobell JM, Zouboulis CC, Gu Y, Williams DA, Sundaram M. HiSCR (Hidradenitis Suppurativa Clinical Response): a novel clinical endpoint to evaluate therapeutic outcomes in patients with hidradenitis suppurativa from the placebo controlled portion of a phase 2 adalimumab study. *J Eur Acad Dermatol Venereol*. 2016;30(6):989-994.
7. Kimball AB, Okun MM, Williams DA, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. *N Engl J Med*. 2016; 375:422-434.
8. InflaRx. InflaRx announces top-line SHINE Phase IIb results for IFX-1 in hidradenitis suppurativa. Available at: https://www.inflarx.de/Home/Investors/Press-Releases/06-2019-InflaRx-Announces--Top-Line-SHINE-Phase-IIb-Results-for-IFX-1-in-Hidradenitis-Suppurativa-.html. Accessed September 10, 2020.
9. Ali AA, Seng EK, Alavi A, Lowes MA. Exploring changes in placebo treatment arms in hidradenitis suppurativa randomized clinical trials: a systematic review. *J Am Acad Dermatol*. 2020;82(1):45-53.

10. von der Werth JM, Williams HC. The natural history of hidradenitis suppurativa. *J Eur Acad Dermatol Venereol*. 2000; 14(5):389-392.
11. Maarouf M, Clark AK, Lee DE, Shi VY. Targeted treatments for hidradenitis suppurativa: a review of the current literature and ongoing clinical trials. *J Dermatolog Treat*. 2018;29(5): 441-449.
12. Bland JM, Altman DG. Measurement error proportional to the mean. *BMJ*. 1996;313(7049):106.
13. R Core Team. R: A language and environment for statistical computing. R Project for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/. Accessed November 3, 2020.
14. Prens EP, Lugo-Somolinos AM, Paller AS, et al. Baseline characteristics from UNITE: an observational, international, multicenter registry to evaluate hidradenitis suppurativa (acne inversa) in clinical practice. *Am J Clin Dermatol*. 2020;21(4):579-590.

4.6: Novel Therapeutics in HS:

Manuscript No	Manuscript Reference
6-1	Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, Ungar J, Krueger JG The Effect of Subcutaneous Brodalumab upon Clinical Disease Activity in Hidradenitis Suppurativa: An Open Label Cohort Study. J Am Acad Dermatol 2020; 83(5):1341-1348
6-2	Frew JW, Navrazahina K, Garcet S, Sullivan-Whalen M, Gilleaudeau P, Krueger JG Weekly Administration of Brodalumab in Hidradenitis Suppurativa: An Open Label Cohort Study. Br J Dermatol 2020; 184(2):350-352

Table 4.6.1

It is well-acknowledged that there is a large unmet need for novel therapeutics in HS[19]. Based on the previously described mechanisms of the Th17 immune axis, IL-17A, Il-17C and IL-17F in the epithelium of HS, it has been proposed that IL-17 antagonism may be a novel therapeutic approach to treating HS[19]. Various monoclonal antibody therapies against different components of the Th17 immune axis exist including Secukinumab and Ixekizumab (IL-17A Monoconal Antibodies), Bimikizumab (IL-17A/F antibody) and Brodalumab (an IL-17RA antagonist)[81]. Brodalumab is unique amongst these agents that it is a receptor antagonist which blocks the action of all IL-17 isoforms (IL-17A, IL-17C and Il-17F) identified in HS epidermal tissue[82]. IL-17RA is also present on fibroblasts, B cells, Neutrophils and various other immune cells which have been implicated in HS[45,46,50,82].

The initial proof of concept study demonstrating a high level of clinical response with Brodalumab (6-1) showed a rapid and sustained reduction in inflammatory nodules and reduction in draining tunnels in select individuals. Heterogeneity was seen between individuals with the presence of draining epithelialised tunnels associated with a lack of sustained clinical

response. This has direct relevance to the mechanistic insights seen in chapter 4.2, particularly publications 2-4 and 2-5.

These observations led to the design and conduct of a second clinical study (6-2) examining an increased frequency of administration of Brodalumab. This increased frequency of administration demonstrated significant benefits in both objective and subjective measurements of disease activity when compared to the standard frequency of administration.

Overall, these open-label clinical trials provide direct clinical relevance as to the role of IL-17RA related pathways in HS, as well as emphasising the impact of epithelialised tunnels upon impacting the efficacy of therapy in the disease.

4.6.1: Publication 6-1

Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, Ungar J, Krueger JG The Effect of Subcutaneous Brodalumab upon Clinical Disease Activity in Hidradenitis Suppurativa: An Open Label Cohort Study. J Am Acad Dermatol 2020; 83(5):1341-1348

The effect of subcutaneous brodalumab on clinical disease activity in hidradenitis suppurativa: An open-label cohort study

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Background: Hidradenitis suppurativa is an autoinflammatory disorder of keratinization, with dysregulation of T helper type 17 cytokines. Brodalumab is a monoclonal antibody that targets the interleukin (IL) 17 receptor A receptor.

Objectives: To assess the safety and tolerability and clinical response at weeks 12 and 24 of brodalumab in moderate to severe HS. Ten participants with no history of inflammatory bowel disease were administered brodalumab 210 mg/1.5 mL subcutaneously at weeks 0, 1, and 2 and every 2 weeks thereafter until week 24. Participants were assessed for adverse events (grade 2/3 adverse events) and clinical response (Hidradenitis Suppurativa Clinical Response [HiSCR], Sartorius, International Hidradenitis Suppurativa Severity Scoring System [IHS4]), including ultrasonography and skin biopsies.

Results: All 10 participants completed the study. No grade 2/3 adverse events associated with the use of brodalumab were reported. All patients (100%) achieved HiSCR, and 80% achieved IHS4 category change at week 12. HiSCR achievement occurred as early as week 2, likely due to the unique blockade of IL-17A, IL-17C, and IL-17F by brodalumab. Significant improvements were seen in pain, itch, quality of life, and depression.

Conclusions: Brodalumab was well tolerated in this HS cohort, with no serious adverse events and improvement in clinical outcomes. Alterations in dose frequency may be required in those with advanced disease, which requires further exploration. (J Am Acad Dermatol 2020;83:1341-8.)

Key words: acne inversa; biologics; brodalumab; cohort study; hidradenitis suppurativa; IL-17A; IL-17C; IL-17F; IL-17RA; monoclonal therapeutics; open label; Th17; translational medicine.

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IRB approval status: Reviewed and approved by Rockefeller University Institutional Review Board.

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Hidradenitis suppurativa (HS) is an autoinflammatory disorder of keratinization[1]; the inflammatory component of the disease involves dysregulation of the T helper (Th) type 17 immune axis.[2] Interleukin (IL) 17A, IL-17C, IL-17F, and IL-23 have all been identified in lesional tissue of patients with HS,[3,4] and a number of IL-17 therapeutic antibodies are currently undergoing clinical trials in HS. However, because of the differential affinity of these agents against different IL-17 isoforms,[5] the relative contributions of each to the inflammatory mechanisms in HS remain unclear.

Brodalumab[6] is an IL-17 receptor (IL-17R) antagonist approved by the US Food and Drug Administration for the treatment of moderate to severe psoriasis.[7,8] Through binding to IL-17RA (part of the IL-17 receptor dimer), it enables blockade of multiple isoforms of IL-17, most pertinently IL-17A, IL-17C, and IL-17F, which are known contributors to inflammation in psoriasis and atopic dermatitis[9] as well as in lesional HS tissue.[4]

CAPSULE SUMMARY

- In this open-label cohort study (N = 10) investigating the effect of subcutaneous brodalumab 210 mg/1.5 mL every 2 weeks on disease activity in hidradenitis suppurativa (HS), no serious adverse effects were reported; 100% of participants (N = 10) achieved Hidradenitis Suppurativa Clinical Response at week 12. Significant improvements were seen in pain, itch, quality of life, and depression, with no episodes of self-harm or suicidality.
- Brodalumab was well tolerated and showed a high rate of clinical response in this cohort. Larger placebo-controlled studies stratifying by disease severity are encouraged to further explore the safety and efficacy of brodalumab in HS.

The objectives of this open-label pilot cohort study were to evaluate the safety and tolerability of brodalumab in participants with HS (as graded by the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 5.0)[10] as well as the effect of brodalumab upon clinical disease activity (if any) in participants with HS. Clinical disease activity was assessed through the use of Hidradenitis Suppurativa Clinical Response (HiSCR),[11] International Hidradenitis Suppurativa Severity Score (IHS4),[12] and Sartorius score.[13] This study was approved by the institutional review board of Rockefeller University and prospectively registered on clinicaltrials.gov (NCT03960268). Reporting was conducted in line with the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) checklist.[14]

METHODS

This study was conducted at The Rockefeller University Hospital, New York, between May 2019 and January 2020. Patients were screened and, if eligible, underwent informed consent discussions in line with the Declaration of Helsinki. Patients older than 18 years with moderate to severe (Hurley stage 2 or 3) HS were eligible for participation. Patients were required to have negative tests results for hepatitis B virus surface antigen, hepatitis C virus antibody, HIV, and tuberculosis (as measured by QuantiFERON Gold) and could not be pregnant or breastfeeding. Individuals with a personal history of inflammatory bowel disease were also excluded from participation.[15] Complete inclusion and exclusion criteria for this study are provided in Supplemental Table I (available via Mendeley at https://data.mendeley.com/datasets/98pmyyz67m/1). Individuals taking a biologic or immunomodulating therapy underwent a washout period of 5 half-lives before enrollment in the trial. Clinical assessments, blood work (routine hematologic values including hemoglobin, leucocyte, and platelet count) and skin biopsies were taken at weeks 0, 4, and 12. Biopsies were performed with the assistance of cutaneous ultrasonography (GE [Boston, MA] Logic Q 20-MHz probe), and biopsy sites (lesional, perilesional, and unaffected) were chosen in line with published recommendations for translational research studies in HS.[16] Brodalumab 210 mg/1.5 mL was administered via a prefilled syringe at weeks 0, 1, and 2 and every 2 weeks thereafter until week 24.

The primary safety evaluation was the number of grade 2/3 adverse events during the 24 weeks of the clinical study. Change in clinical disease activity was assessed by the number of patients achieving HiSCR[11] (defined as a ≥50% reduction in inflammatory lesion count—abscesses plus inflammatory nodules—and no increase in abscesses or draining fistulas) at week 12 and week 24 compared with baseline; as well as change in the Sartorius score[13] and the IHS4[12] at weeks 12 and 24 compared with baseline. Patient-reported outcomes including Visual Analogue Scales of pain, itch, and global disease assessment; Dermatology Life Quality Index[17] (DLQI); Beck Depression Inventory[18] (BDI); and Patient Health Questionnaire-9[19] were administered at weeks 0, 12, and 24.

Abbreviations used:

AN:	abscess and nodule count
BDI:	Beck Depression Inventory
DLQI:	Dermatology Life Quality Index
HiSCR:	Hidradenitis Suppurativa Clinical Response
HS:	hidradenitis suppurativa
IHS4:	International Hidradenitis Suppurativa Severity Score
IL:	interleukin
IL-17R:	interleukin 17 receptor
Th:	T helper

Statistical analyses of safety and tolerability were analyzed descriptively. Changes in clinical outcomes and patient-reported outcomes were analyzed using nonparametric assessments for paired assessments (Wilcoxon's matched-pairs signed rank test) with correction for multiple comparisons. Missing data was handled using nonresponder imputation.

RESULTS

Demographic characteristics of included patients are presented in Supplemental Table II (available via Mendeley at https://data.mendeley.com/datasets/98pmyyz67m/1). Overall, 50% of participants were male, and 50% were female, with 6 out of 10 patients being active smokers. Eight of the 10 participants had Hurley stage 2 disease with a median abscess and nodule count (AN count) of 10 (range, 4-16) and median draining fistula count of 3 (range, 0-35). Six of the 10 patients were obese (body mass index, >30.0 kg/m^2), and previous therapies included oral antibiotic therapies (n = 10), adalimumab (n = 7), infliximab (n = 4), secukinumab (n = 2), ixekizumab (n = 2), and large surgical excisions with HS recurrence (n = 6).

All 10 patients completed the 2 primary timepoints of week 12 and week 24. A 12-week observation period was designed after the completion of the 24-week treatment period to assess response after treatment withdrawal; however, all participants withdrew from the study at week 24 to avoid the 12-week treatment withdrawal period. All patients elected to continue with brodalumab therapy after the completion of the study.

All patients (100%) achieved HiSCR at week 2 compared with baseline (Fig 1), with 5 of 10 patients achieving a 75% reduction in AN count and 3 of 10 patients achieving a 100% reduction in AN count. All 10 patients (100%) had achieved HiSCR at week 12, with 7 of 10 patients achieving a 75% reduction in AN count, and 40% of patients achieving a 100% reduction in AN count (Fig 1). This continued to increase, with 100% patients having a 75% reduction in AN count at week 24 and 40% of patients having a 100% response in AN count. At week 2, 50% patients achieved IHS4 category change, increasing to 80% at week 12, which was maintained until week 24. At week 12, 40% of patients had a 2-category change in IHS4 score that was maintained until week 24.

Average nodule counts, draining tunnel counts, and IHS4 scores all reduced dramatically within the first 2 weeks of therapy (Fig 2), with an increase in draining tunnel counts at week 4, which then continued to decrease over time. Pain and Itch Visual Analogue Scale scores steadily decreased (Fig 2), which mirrored the steady decreases in patient-reported outcomes such as overall disease severity, DLQI, BDI, and PHQ-9 scores (Fig 3). Total Sartorius scores significantly decreased at weeks 12 and 24 compared to baseline in all participants. The changes in nodule, draining tunnel, IHS4, and pain and itch scores were statistically significant compared to baseline at all timepoints (Fig 2). The changes in disease severity, DLQI, and PHQ-9 scores were statistically significant from baseline at weeks 12 and 24, with the changes in BDI scores significant from baseline only at the week 24 timepoint (Fig 3).

Significant decreases in vascularity and inflammation, as measured by cutaneous Doppler ultrasonography, were seen at weeks 4, 12, and 24 compared with baseline in all 10 patients (Fig 4). Doppler signal reductions were particularly apparent in the superficial dermis and surrounding parallel hyperechoic structures of the dermis, indicative of epithelialized tunnels (Fig 4, *A*, *B*, *E*, and *F*).

Reduction in psoriasiform epidermal hyperplasia, with re-establishment of a granular layer, reduction in parakeratosis, and CD3$^+$ and CD11c dermal infiltrates were also noted at weeks 4, 12, and 24 compared with baseline (Fig 5). These changes were seen in lesional, perilesional, and unaffected skin samples across all participants.

DISCUSSION

No serious adverse events were noted in the setting of brodalumab therapy in this HS cohort. The 4 adverse events reported (Supplemental Table III, available via Mendeley at https://data.mendeley.com/datasets/98pmyyz67m/1) were minor, with 3 of the 4 events unlikely to be associated and the 1 self-resolving upper respiratory tract infection possibly associated, given the reports of upper respiratory tract infection in the phase 3 randomized controlled trial of brodalumab in psoriasis.[8]

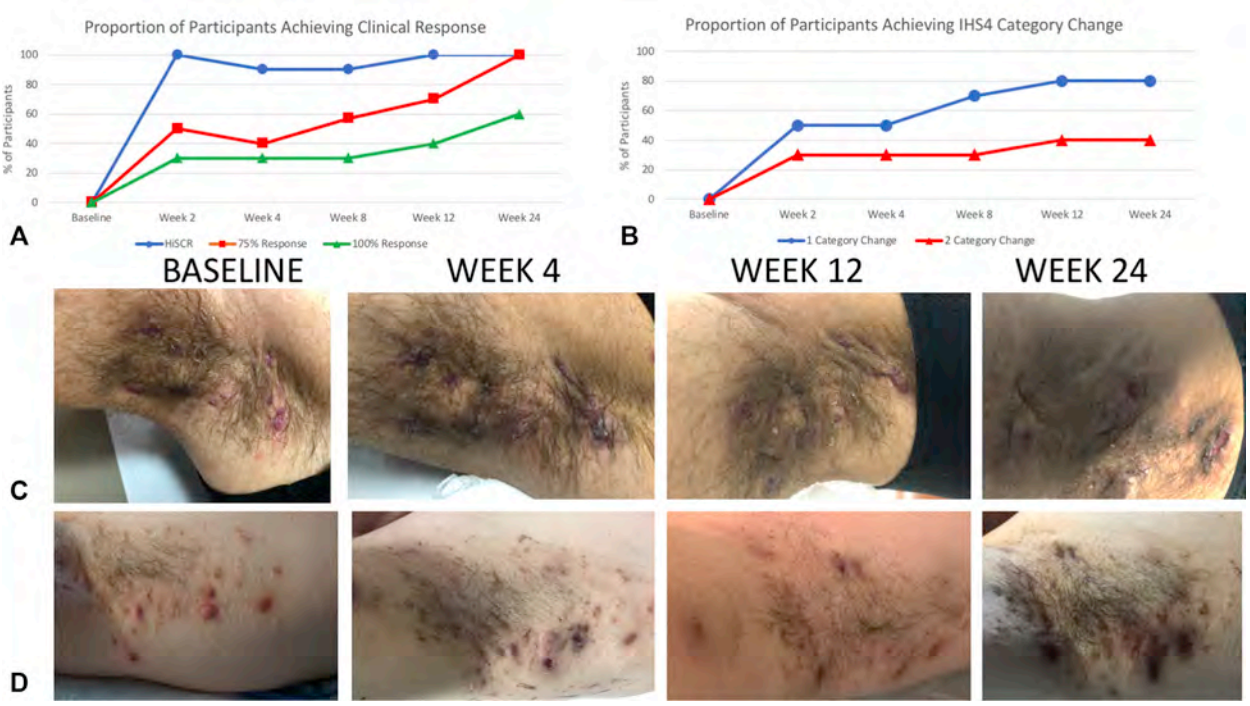

Fig 1. Measures of clinical response to brodalumab therapy in hidradenitis suppurativa. **A**, The proportion of patients achieving HiSCR at weeks 12 and 24 was 100%, with 75% reduction in AN counts (red) and 100% reduction in AN counts (green) in 60% and 40% of participants, respectively. **B**, Measurement of clinical outcomes using IHS4 category change shows 80% and 40% of patients achieving 1-category and 2-category change respectively. **C** and **D**, Representative clinical photos show a rapid reduction in the inflammatory nature of nodules at week 4 compared to baseline and continued improvement at week 12. HiSCR was maintained at Week 24 despite external triggers initiating limited flares of disease. *AN*, Abscess and nodule count; *HiSCR*, Hidradenitis Suppurativa Clinical Response; *IHS4*, International Hidradenitis Suppurativa Severity Score.

The rapid reduction in inflammatory lesion count (AN count) was unexpected but consistent with the mechanism of action of brodalumab as an IL-17RA antagonist acting on a number of different cell types, including neutrophils, dendritic cells, keratinocytes, and other inflammatory leucocytes.[5] The ability of brodalumab to block IL-17A, IL-17C, and IL-17F may be important because each of these cytokines can drive neutrophilic inflammation,[7] with the blockade of all 3 isoforms a potential benefit above other agents blocking only IL-17A or IL-17A/IL-17F. Other IL-17 monoclonal antibodies trialed in HS do not have the ability to block the range of IL-17 isoforms possible with brodalumab. The pharmacodynamic properties of brodalumab in suppressing the downstream cascade of keratinocyte-derived CXCL cytokines and other inflammatory mediators has been observed in psoriasis,[7] and verification of brodalumab's similar mechanism of action in HS is required. If verified, this would lend further credence to the suggestion of HS being an autoinflammatory disease of keratinocytes.[1]

The pronounced reduction in cutaneous inflammation is visible upon clinical examination (Fig 1), although larger nodules and deeper abscesses may take longer than 12 weeks to resolve (along with any residual postinflammatory hyperpigmentation). The rapid reduction in pain (Fig 2) and steady improvement in patient-reported outcomes (Fig 3) such as the DLQI are consistent with the known PD properties of this drug in suppressing Th17-mediated inflammatory pathways.[7] Two participants with severe, widespread Hurley stage 3 disease had greater pain levels during the 24 weeks of treatment, despite clinical improvement, and this may be explained by a degree of central pain sensitization in the setting of severe disease.[20]

What is not able to be appreciated from the clinical photographs is the reduction in dermal

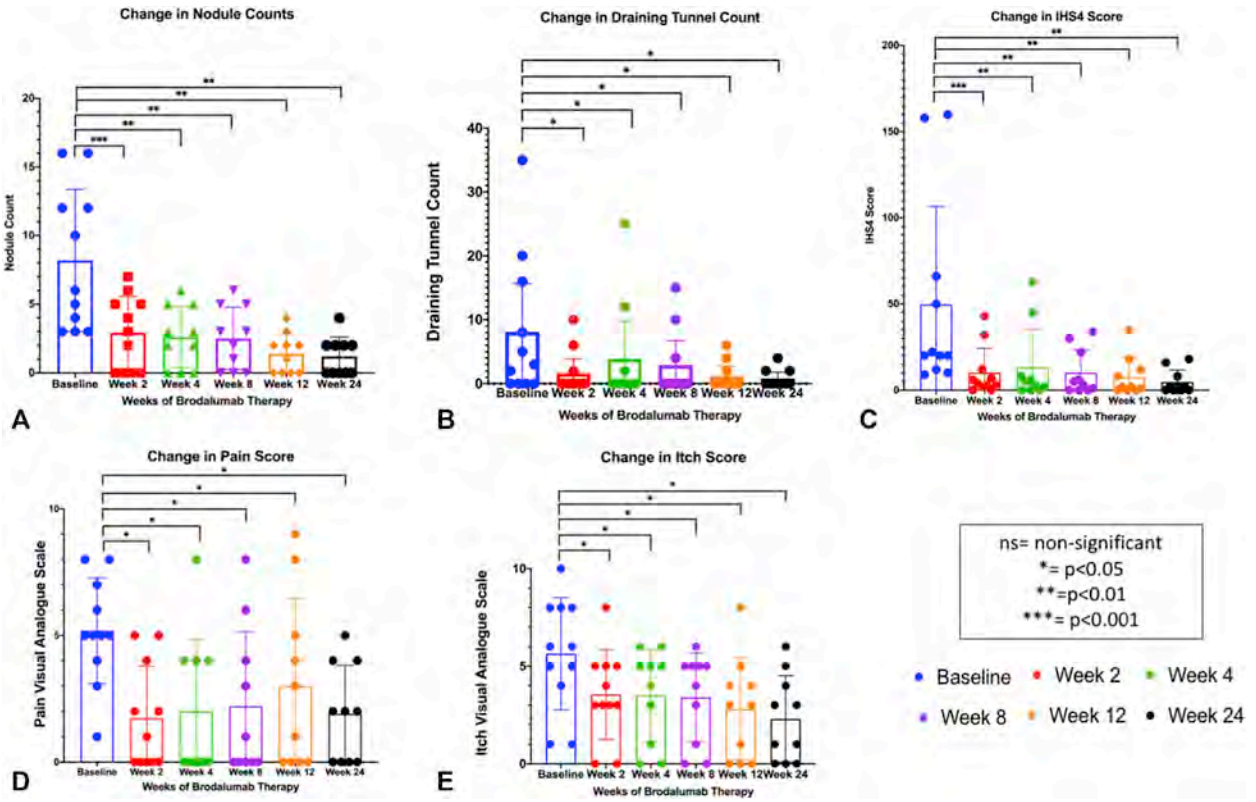

Fig 2. Brodalumab therapy shows significant reductions in (**A**) mean nodule count, (**B**) mean draining tunnel count, (**C**) mean IHS4 score, (**D**) mean pain numerical score, and (**E**) mean itching numerical score. Each outcome was statistically significant from baseline at weeks 12 and 24 with average nodule count, average IHS4 score, average pain score, and average itch score being statistically significant at week 2 of therapy. *IHS4*, International Hidradenitis Suppurativa Severity Score.

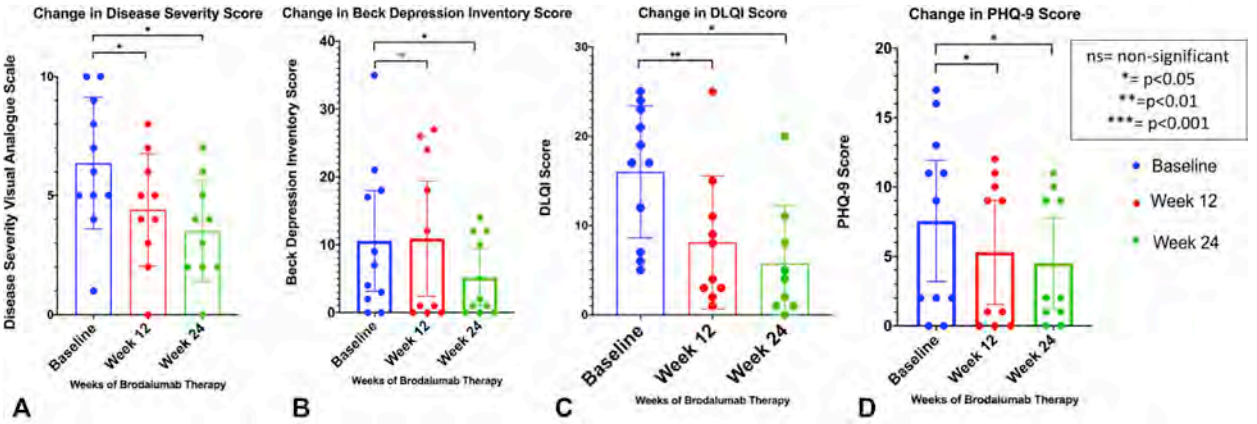

Fig 3. Patient-based outcome measures were significantly reduced at weeks 12 and 24 of brodalumab therapy compared with baseline scores. Patient-rated disease severity (**A**) Visual Analogue Scale scores, (**B**) mean DLQI scores, (**C**) mean Beck Depression Inventory scores, and (**D**) Mean PHQ-9 scores were reduced significantly at weeks 12 and 24. *DLQI*, Dermatology Life Quality Index; *PHQ-9*, Patient Health Questionnaire-9.

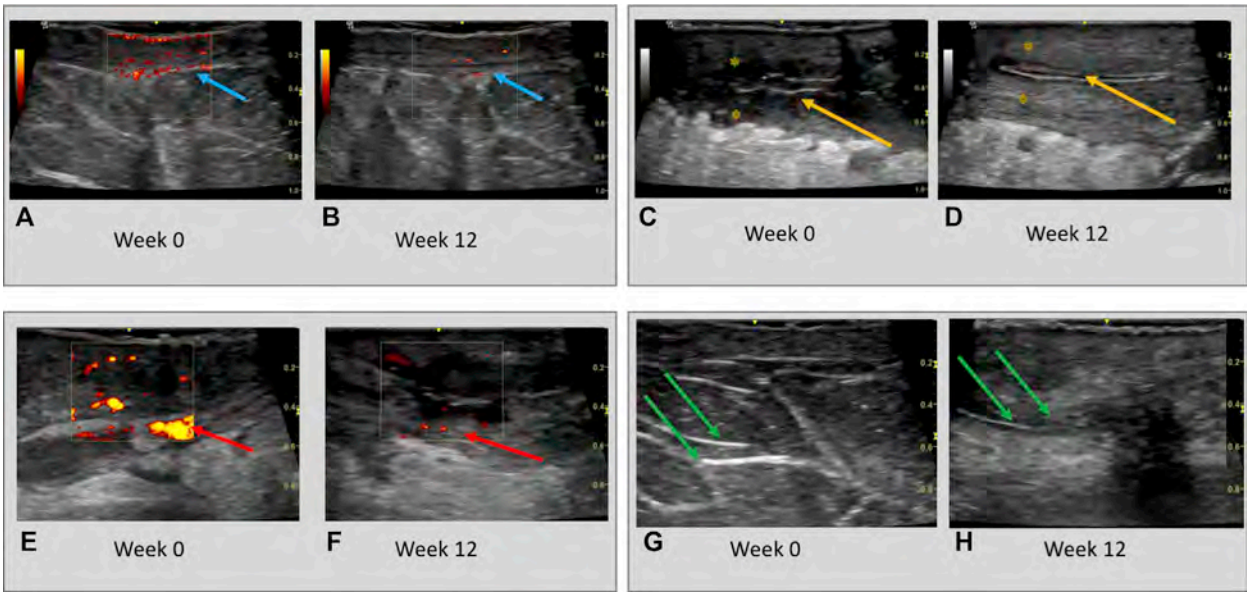

Fig 4. **A**, **C**, **E**, and **G**, Ultrasonographic changes at baseline and (**B**, **D**, **F**, **H**) after 12 weeks of treatment with brodalumab. Dermal Doppler ultrasonography intensity in lesional dermal tissues at (**A**) baseline (blue arrows) is significantly attenuated at (**B**) week 12 (blue arrows) of therapy. The diameter of draining tunnels (yellow arrows) and surrounding edema (yellow asterisk) at (**C**) baseline is also reduced at (**D**) week 12. Similarly, Doppler intensity (red arrows) surrounding (**E**) epithelialized dermal tunnels (**F**) reduces at week 12 compared with baseline. The diameter and echogenicity of dermal tunnels (green arrows) is also (**H**) attenuated at week 12 compared with (**G**) baseline.

tunnel drainage after administration of brodalumab. Lesional edema, tenderness, and drainage were reduced significantly during the loading doses of brodalumab (weeks 0, 1, and 2) and after drug administration throughout the remainder of the 24 weeks (Supplemental Fig 4, available via Mendeley at https://data.mendeley.com/datasets/98pmyyz67m/1). This suggests that the action of brodalumab not only targets the development of cutaneous nodules and abscesses but also the purulent discharge from epithelialized dermal tunnels. This is supported by the results of decreased dermal Doppler ultrasonographic intensity surrounding tunnels and the reduction in the diameter and hyperechoic intensity of tunnels after Brodalumab therapy. The suggestion that epithelialized tunnels may be active contributors to inflammation is a concept that requires further mechanistic evaluation.

A black box warning exists in the United States regarding the association between brodalumab and suicidality, although the causation has been disputed in the literature.[21] In the design of this pilot study, we astutely monitored each patient's mental state at each visit and via telephone contact in between visits and had no occurrences of suicidal thoughts or behaviors. In fact, patient-reported depression scores (BDI and PHQ-9) were significantly reduced at week 24 compared to baseline, with PHQ-9 also significantly reduced at week 12. Despite the small sample size, this lends credence to the suggestion of Lebwohl et al[21] (supported by observational evidence[22]) that untreated or insufficiently treated cutaneous disease is a large contributor to depression and suicidality.

The fact that 100% of patients achieved HiSCR at week 2 of this study emphasizes the response of this cohort to brodalumab therapy. Given the documented concerns regarding elevated placebo response rates in HS clinical trials[23,24] and the results of this and other cohort studies of IL-17 and IL-23 inhibitors in HS,[25-28] we undertook a deeper analysis of clinical response through evaluation of the 75% and 100% response rates in this cohort, showing a progressive reduction in clinical disease throughout the 24 weeks of the study. In a similar way to how reductions in Psoriasis Area and Severity Index scores of 50%,75%, and 90% have been surpassed in psoriasis, more comprehensive measures of clinical response may need to be adopted in HS. The additional complicating factor in HS, however, is that the morphologic heterogeneity of disease (nodules, tunnels, etc), means that the IHS4 may have advantages over nodule counts, given the inclusion of draining tunnels in its scoring.[24] The

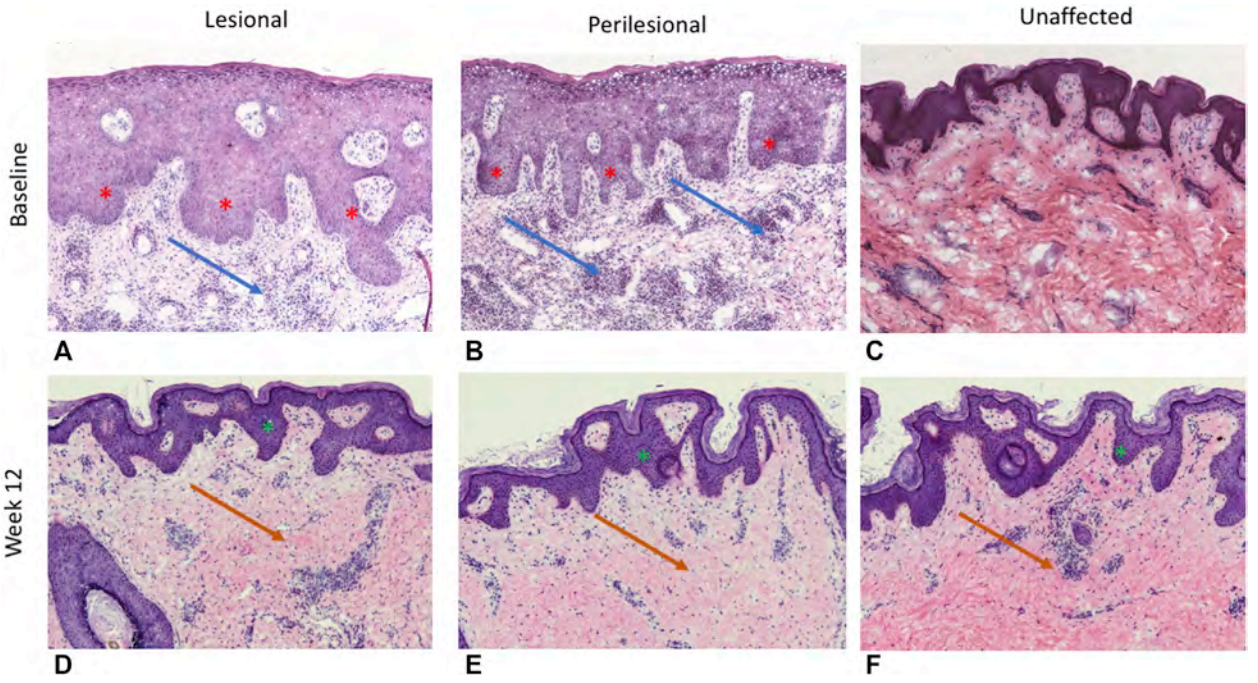

Fig 5. Representative histology of (**A**, **D**) lesional, (**B**, **E**) perilesional, and (**C**, **F**) unaffected tissue at (**A**-**C**) baseline (week 0) and (**D**-**F**) week 12 of treatment with brodalumab 210 mg/1.5 mg every 2 weeks. Baseline lesional and perilesional samples illustrate significant psoriasiform epidermal hyperplasia (red asterisk) with diffuse mixed dermal inflammatory infiltrate (blue arrows). Unaffected tissue illustrates milder manifestations of inflammatory infiltrates and psoriasiform epidermal hyperplasia. After 12 weeks of brodalumab therapy, psoriasiform epidermal hyperplasia is much less pronounced (green asterisk), with a noticeable reduction in dermal inflammatory infiltrates (orange arrows).

response rate in this study may be influenced by the fact that 8 of the 10 participants had Hurley stage 2 disease, with 5 participants with dermal tunnels on ultrasonography and histology. Given the influence of tunnels on clinical response rates with adalimumab,[24] it is not inconceivable that stratification of severity by the presence of tunnels may be required in larger trials to accurately assess the impact of Brodalumab on Hurley stage 3 disease.

Two patients had widespread, severe Hurley stage 3 disease (patients 2 and 6) (Supplementary Table II), and although these patients achieved HiSCR, the response of their draining tunnels was cyclic. Tunnels would reduce and/or cease draining within 24 hours of dose administration, and the greatest sustained improvement was seen during the loading dose period (weeks 0-3). Once on the every-2-week dosing regimen, however, 5t o 7 days after dosing, the patient would report extensive painful drainage, suggesting an insufficient frequency of dosing. Although overall the trend over 24 weeks was toward improvement, these patients represent anecdotal evidence that in severe disease with extensive dermal tunnels, an increased frequency of dosing (as in recalcitrant psoriasis[29]) may need to be explored.

This study is limited by its lack of placebo control arm, size (N = 10), and short duration of therapy (24 weeks). It is comparable in size to studies involving anakinra[30] and infliximab,[31] but larger cohorts, longer trials, and a variety of dosing frequencies are suggested as considerations for future studies, given the positive response of this pilot cohort.

CONCLUSION

Brodalumab administered over 24 weeks in this pilot cohort did not result in any severe adverse events. Clinical response to brodalumab therapy was rapid, with 100% of the cohort achieving HiSCR at weeks 12 and 24. No safety signals regarding depression or suicidality were identified. Alterations in dosing frequency may be required in future studies to provide sustained effect in participants with extensive draining tunnels. Larger placebo-controlled studies are required to establish the true potential of brodalumab as an effective treatment for HS.

All brodalumab used in this study was provided under an Investigator Initiated Study Agreement with Valeant Pharmaceuticals. Valeant Pharmaceuticals had no input into the conduct of the study, analysis of the data, or composition of the manuscript.

REFERENCES

1. De Vita V, McGonagle D. Hidradenitis suppurativa as an autoinflammatory keratinization disease. *J Allergy Clin Immunol*. 2018;141(56):1953.
2. Moran B, Sweeney CM, Hughes R, et al. Hidradenitis suppurativa is characterized by dysregulation of the Th17:Treg cell axis which is corrected by anti-TNF therapy. *J Invest Dermatol*. 2017;137(11):2389-2395.
3. Witte-Handel E, Wolk K, Tsaousi A, et al. The IL-1 pathway is hyperactive in hidradenitis suppurativa and contributes to skin infiltration and destruction. *J Invest Dermatol*. 2019;139(6):1294-1305.
4. Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. *Br J Dermatol*. 2020;182:1045-1047.
5. Gaffen S. IL-7 receptor composition. *Nat Rev Immunol*. 2016;16:4.
6. Papp K, Leonardi C, Menter A, et al. Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. *N Engl J Med*. 2012;366:1181-1189.
7. Russell CB, Rand H, Bigler J, Kerkof K, Timour M, Bautista E. Gene expression profiles normalized in psoriatic skin by treatment with brodalumab, a human anti-il-17 receptor monoclonal antibody. *J Immunol*. 2014;192:3828-3836.
8. Papp K, Reich K, Paul C, et al. A prospective phase III, randomized, double-blind, placebo controlled study of brodalumab in patients with moderate-to-severe plaque psoriasis. *Br J Dermatol*. 2016;175:273-286.
9. Guttman-Yassky E, Krueger JG. atopic dermatitis and psoriasis: two different immune diseases or one spectrum? *Curr Opin Immunol*. 2017;48:68-73.
10. US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE v5.0). November 27, 2017. Available at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50. Accessed June 8, 2020.
11. Kimball AB, Sobell JM, Zouboulis CC, et al. HiSCR (Hidradenitis Suppurativa Clinical Response): a novel clinical endpoint to evaluate therapeutic outcomes in patients with hidradenitis suppurativa from the placebo-controlled portion of a phase 2 adalimumab study. *J Eur Acad Dermatol Venereol*. 2016;30(6):989-994.
12. Zouboulis CC, Tzellos T, Kyrgidis A, et al. Development and validation of the International Hidradenitis Suppurativa Severity Score System (IHS4), a novel dynamic scoring system to assess HS severity. *Br J Dermatol*. 2017;177(5):1401-1409.
13. Sartorius K, Emtestam L, Jamac GB, Lapins J. Objective scoring of hidradenitis suppurativa reflecting the role of tobacco smoking and obesity. *Br J Dermatol*. 2009;161(4):831-839.
14. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-349.
15. Targan SR, Feagan B, Vermeire S, et al. A randomized, double blind, placebo-controlled phase 2 study of brodalumab in patients with moderate-to-severe Crohn's disease. *Am J Gastroenterol*. 2016;111(11):1599-1607.
16. Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR, Kirby JS. Defining lesional, perilesional and unaffected skin in hidradenitis suppurativa: proposed recommendations for clinical trials and translational research studies. *Br J Dermatol*. 2019;181(6):1339-1341.
17. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)—a simple practical measure for routine clinical use. *Clin Exp Dermatol*. 1994;19(3):210-216.
18. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-571.
19. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606-613.
20. Neilsen RM, Andersen PL, Sigsgaard V, Mikkelsen PR, Jemec GBE. Pain perception in patients with hidradenitis suppurativa. *Br J Dermatol*. 2020;182:166-174.
21. Lebwohl MG, Papp KA, Marangell LB, Koo J, Blauvelt A, Gooderham M. Psychiatric adverse events during treatment with brodalumab: analysis of psoriasis clinical trials. *J Am Acad Dermatol*. 2018;78:81-89.
22. Machado MO, Stergiopoulos V, Maes M, Kurdyak PA, Lin PY, Wang LJ. Depression and anxiety in adults with hidradenitis suppurativa: a systematic review and meta-analysis. *JAMA Dermatol*. 2019;155(8):939-945.
23. Ali AA, Seng EK, Alavi A, Lowes MA. Exploring changes in placebo treatment arms in hidradenitis suppurativa randomized clinical trials: a systematic review. *J Am Acad Dermatol*. 2020;82(1):45-53.
24. Frew JW, Jiang CS, Singh N, et al. Clinical response rates, placebo response rates and significantly associated covariates are dependent upon choice of outcome measure in hidradenitis suppurativa: a post-hoc analysis of PIONEER 1 and 2 individual patient data. *J Am Acad Dermatol*. 2020;82:1150-1157.
25. Kovacs M, Podda M. Guselkumab in the treatment of severe hidradenitis suppurativa. *J Eur Acad Dermatol Venereol*. 2019;33(3):e140-e141.
26. Casseres RG, Kahn JS, Her MJ, Rosmarin D. Guselkumab in the treatment of hidradenitis suppurativa: a retrospective chart review. *J Am Acad Dermatol*. 2019;81(1):265-267.
27. Marasca C, Megna M, Balato A, Balato N, Napolitano M, Fabbrocini G. Secukinumab and hidradenitis suppurativa: friends or foes? *JAAD Case Rep*. 2019;5(2):184-187.
28. Odorici G, Pellacani G, Conti A. Ixekizumab in hidradenitis suppurativa: a case report in a psoriatic patient [e-pub ahead of print]. *G Ital Dermatol Venereol*. https://doi.org/10.23736/S0392-0488.18.06135-7. Accessed June 8, 2020.
29. Yeung J, Ladda M, Piguet V. Dose optimisation of brodalumab in moderate-to-severe plaque psoriasis. Abstract presented at: 24th World Congress of Dermatology; June 10-15, 2019. Milan, Italy.
30. Tzanetakou V, Kanni T, Giatrakou S, Katoulis A, Papdavid E, Netea MG. Safety and efficacy of anakinra in severe hidradenitis suppurativa: a randomized clinical trial. *JAMA Dermatol*. 2016;152(1):52-59.
31. Grant A, Gonzales T, Montgomery MO, Cardenas V, Kerdel FA. Infliximab therapy for patients with moderate to severe hidradenitis suppurativa: a randomized, double-blind, placebo-controlled crossover trial. *J Am Acad Dermatol*. 2020;62(2):205-217.

4.6.2: Publication 6-2

Frew JW, Navrazahina K, Garcet S, Sullivan-Whalen M, Gilleaudeau P, Krueger JG Weekly Administration of Brodalumab in Hidradenitis Suppurativa: An Open Label Cohort Study. Br J Dermatol 2020; 184(2):350-352

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Article Available on Publisher Website:
https://onlinelibrary.wiley.com/doi/10.1111/bjd.19478

CHAPTER 5: HOW THE PRESENTED PUBLICATIONS ARE LINKED:

All of the publications included in this dissertation focus upon the disease Hidradenitis Suppurativa. Chapter 1 focuses on the existing body of knowledge regarding the molecular pathogenesis of the disease. Chapter 2 critically examines the potential roles of other mechanisms which have theoretical foundations in playing a role in the pathogenesis of the disease. Chapter 3 integrates the existing knowledge regarding the genetics of HS and uses in silico techniques to test hypotheses regarding the genetic underpinnings of polymorphisms in the gamma secretase complex in the pathogenesis of HS. Chapter 4 then takes individual patient level data from Phase 3 clinical trials and re-analyses this data to examine the role of various clinical manifestations upon the efficacy of TNF-alpha antagonism in HS. Chapter 5 then presents investigations and analyses of how clinical trials and mechanistic investigations in HS are designed and implemented in order to highlight current deficiencies in HS clinical trials and provide evidence-based solutions to address these issues. The final chapter (Chapter 6) then presents the results of two open-label clinical trials using a novel therapeutic in HS – Brodalumab – and presents data regarding the clinical efficacy of this intervention in the setting of HS.

As a coherent body of work the publications in this dissertation summarise, critically evaluate, and present new analyses of existing data pertaining to the pathogenesis and clinical management of HS. Additionally, several publications present novel data regarding the pathogenesis of HS, the clinical relevance of disease morphology to disease activity, as well as clinical evidence regarding a novel therapeutic agent (Brodalumab).

CHAPTER 6: OVERALL SIGNIFICANCE, LIMITATIONS AND FUTURE DIRECTIONS:

6.1 Significance of Presented Work: Bibliometric Data

The significance of publications included in this dissertation can be assessed using citations, as well as metrics of engagement via internet searches, internet views/downloads and discussion on social media platforms since publication. These can be quantified and objectively assessed through bibliometrics and altimetrics[83]. Bibliometrics are used to explore the impact of certain authors or publications using methods including citation analysis, impact factor or h-index. Altmetrics are considered 'non-traditional' forms of bibliometrics which can often complement the aforementioned methods by examining attention and/or engagement regarding specific authors or publications.

6.1.1 Article-level Bibliometric Data

Bibliometric data for each publication presented in this dissertation up to and including the date 30/06/2021 are included in table 6-1-1 below. The included publications have attained a total of 127 citations via Web of Science and 239 citations via google scholar over a period of 30 months since the first publication release. Additionally, 16 of the 23 included publications have documented discussion in news outlets and social media platforms (including twitter) as indicated via Altmetrics.

No.	Date of Publication	Bibliometric Measures (as of 30th June 2021)							
		Web of Science (citations)	Google Scholar (citations)	ResearchGate			Altmetrics		
				Views	Citations	Recommendations	Score	Twitter	Mendeley
1-1	Dec 2018	NL	36	99	35	1	3	6	17
1-2	Dec 2018	NL	11	42	8	0	6	10	12
1-3	March 2019	18	31	2825	28	1	4	9	49
2-1	Aug 2019	9	12	141	13	0	1	0	20
2-2	Jan 2020	8	9	113	9	1	0	0	33
2-3	May 2020	1	1	56	2	0	2	5	10
2-4	Feb 2021	3	6	86	4	0	1	2	7
2-5	March 2021	NL	2	32	2	1	2	2	2
2-6	July 2020	NL	2	245	3	1	6	7	6
3-1	Oct 2017	33	50	228	42	0	2	4	45
3-2	Sept 2019	12	20	32	38	0	0	0	14
3-3	Sept 2019	2	3	74	4	1	11	3	9
3-4	Feb 2019	2	5	51	3	0	1	1	6
3-5	April 2020	1	4	62	4	0	0	0	8
4-1	March 2021	2	6	59	4	1	1	1	5
4-2	June 2021	NL	0	12	0	0	1	1	0
4-3	Oct 2020	3	3	40	3	0	0	0	8
5-1	Dec 2019	7	13	75	10	0	2	4	16
5-2	May 2020	15	17	166	18	0	2	3	14
5-3	August 2020	2	1	33	2	0	1	3	1
5-4	Dec 2020	NL	0	40	0	0	0	0	0
6-1	Nov 2020	9	15	107	15	0	7	10	16
6-2	Feb 2021	0	2	62	2	0	0	0	5

Table 6-1-1: Tabulated Details of Bibliometric and Altmetric statistics for the publications presented in this dissertation. NL= Not Listed

6.1.2 Journal Level Bibliometric Data

The relevance and impact of the included publications in the field is also indicated by the journal-level bibliometric data. This gives an indication of the journal ranking within the field of dermatology as well as impact factor and citation index to give an indication of the distribution and circulation of the included publications.

Ten of the included publications were published in journals ranked in the top 4 of international dermatology journals globally (Table 6-1-2). Publication 2-4 was published in the Journal of Allergy and Clinical Immunology, ranked 15th in the field of immunology. 14 of the publications were in journals with impact factors greater than 5.00, with 21/23 included publications in journals with a 2020 journal citation indicator greater than 1.00 (Table 6-1-2). This indicates that the majority of these publications were present in journals that reported a greater than average number of citations in the field. Two journals (JAAD International – Publications 2-6 and 5-4, and F1000 Research- Publications 1-1 and 1-2) were not listed on Web of Science and hence comparable Impact factors and Journal Citation Indicators could not be computed for those articles. The impact of these articles are represented by the article level metrics above.

Journal (Category)	Journal Rank				Impact Factor				Journal Citation Indicator			
	2017	2018	2019	2020	2017	2018	2019	2020	2017	2018	2019	2020
Journal of Allergy and Clinical Immunology *(Immunology)*	6/155	6/158	11/159	15/162	13.25	14.11	10.22	10.79	2.93	2.97	2.51	2.35
Journal of the American Academy of Dermatology *(Dermatology)*	2/64	2/66	1/68	1/68	6.89	7.10	8.27	11.52	2.65	2.67	2.83	2.96
Journal of Investigative Dermatology *(Dermatology)*	3/64	4/66	3/68	4/68	6.44	6.29	7.14	8.55	2.69	2.52	2.65	2.51
British Journal of Dermatology *(Dermatology)*	4/64	3/66	4/68	3/68	6.12	6.71	7.00	9.31	2.02	2.30	2.44	2.45
Therapeutic Advances in Chronic Disease *(Pharmacology & Pharmacy)*	20/261	36/267	51/271	60/275	4.90	4.45	4.25	5.09	1.01	0.90	0.96	0.86
Frontiers in Medicine *(Medicine, General and Internal)*	N/A	32/160	29/165	28/169	N/A	3.11	3.90	5.09	0.70	0.88	1.06	1.09
Experimental Dermatology *(Dermatology)*	19/64	19/66	15/68	19/68	2.60	2.86	3.36	3.96	1.25	1.26	1.30	1.32
Clinical and Experimental Dermatology (Dermatology)	44/64	38/66	38/68	25/68	1.48	1.77	1.97	3.47	0.65	0.66	0.64	0.76

Table 6-2-1: Journal Level Metrics for the Journals in which the compiled articles have been published. Journals in which manuscripts have been published with Journal Rank, Impact Factor and Citation Indices over the years in which the compiled manuscripts were published. N/A= Not Available

6.2 Limitations and Scope for Future Works

The included publications have a number of limitations inherent to the type of studies employed and small sample sizes used. In the setting of systematic reviews (1-1,1-2, 1-3) publication bias is a major limitation to interpretation of the data. Negative results and those not in keeping with the accepted literature are less likely to be published. Additionally, in the setting of a systematic review- only the "*known unknowns*" can be identified and discussed. Novel data is needed to explain the reasons underlying such observations. A pertinent example is regarding inflammatory mediators in HS, the heterogeneity of inflammation in previous studies was identified in publications 1-1 and 1-2. However, the reasons underlying such heterogeneity were not appreciated until the publications 2-S1 and 2-S2 presented novel data regarding the inflammatory nature of epithelialized tunnels to potentially explain such heterogeneity.

Publications 2-1, 2-2 and 2-3 identify and discuss novel hypotheses pertaining to the pathogenesis of HS. Limitations to these publications are that they are supported by data in other disease entities, but not validated in the setting of HS. It is important to note however, that since the publication of these articles, a number of the published hypotheses (pertaining to the role of complement in draining epithelialised tunnels and the role of B cells) are currently being explored by a number of research groups and pharmaceutical companies. Specifically, clinical trials targeting aspects of the complement system have been seen to be clinically and statistically significant only in severe tunnel-associated disease[83,84], and B cells are established to play a strong role in severe longstanding HS[85]. The novel mechanistic insights presented in publications

2-S1 and 2-S2 are based on relatively low sample sizes and independent validation would be needed in independent cohorts.

Regarding the genetics of HS, (3-1, 3-2, 3-3, 3-4, 3-5) although the data and interpretation of the role of Notch signalling was based upon a systematic review and in-silico studies, the hypotheses of pathophysiological heterogeneity has been validated in independent experimental studies[73]. The study by Hessam et al[73] demonstrating that the Notch-PI3K-AKT pathways are not modulated in European HS cohorts[73] in the same way observed in East Asian Kindred reported by Xiao et al[71]. More mechanistic work is required to examine the other GSC substrates identified in 3-3 as well as further work through GWAS in the field.

The insights provided in 4-1, 4-2, 4-3 are limited by the assumption that the original data collated in the PIONEER studies is valid and reliable. Similar post-hoc analyses in other clinical trials (using IL-17, IL-23 and complement antagonists) would be of interest to see whether these covariates are disease-associated or therapy specific. These publications have already raised interest in how clinical trials are designed- and if there is a need to stratify trials by disease severity or epithelialised tunnels. As previously mentioned, despite the lack of a positive primary outcome in a Phase 2 study; Phase 3 studies with Avacopan (a complement C5aR1 antagonist) are planned for Hurley Stage 3 HS. Also, inclusion criteria in almost all future HS studies have now been raised to include >5 inflammatory lesions, with the IHS4 and HASI used as secondary outcome measures, based on the published results of publications 5-2 and 5-4.

The preliminary results from 6-1 and 6-2 were limited by their small sample sizes and lack of placebo control groups, however the premise of using other outcome measures alongside the HiSCR has been embraced by many ongoing and future planned clinical trials.

Conclusion:

In summary, the presented publications in this dissertation have provided much needed critical evaluation of the current pathogenic paradigm of HS. Objective bibliographic data demonstrate the wide reach of these publications in the limited time frame since publication. A number of the novel interpretations and hypotheses, particularly regarding complement, B cells and the genetic underpinnings of the disease have been validated and investigated in independent mechanistic studies. Additionally, the mechanistic insights into the immunological role and clinical importance of epithelialised tunnels has made a major impact in the understanding of the heterogeneity of the disease, as well as how targeting of future therapeutics should proceed. Additionally, the way in which future HS clinical trials are designed and implemented has been fundamentally altered by the results of the data from these publications. This dissertation presents work which alters the pathogenic paradigm of HS and lays the foundation for the identification and evaluation of future novel therapeutic targets in the disease.

BIBLIOGRAPHY:

1) Sabat R, Jemec GBE, Matusiak Ł, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. Nat Rev Dis Primers. 2020 Mar 12;6(1):18. doi: 10.1038/s41572-020-0149-1. PMID: 32165620.
2) Verneuil Études sur les tumeurs de la peau; de quelques maladies des glandes sudoripares Arch. Gen. Med., 4 (1854), pp. 447-468
3) Nguyen TV, Damiani G, Orenstein LAV, Hamzavi I, Jemec GB. Hidradenitis suppurativa: an update on epidemiology, phenotypes, diagnosis, pathogenesis, comorbidities and quality of life. J Eur Acad Dermatol Venereol. 2021 Jan;35(1):50-61. doi: 10.1111/jdv.16677
4) Erin K. Collier, Ram K. Parvataneni, Michelle A. Lowes, Haley B. Naik, Martin Okun, Vivian Y. Shi, Jennifer L. Hsiao, Diagnosis and management of hidradenitis suppurativa in women, American Journal of Obstetrics and Gynecology,Volume 224, Issue 1,2021, Pages 54-61,
5) Liy-Wong C, Kim M, Kirkorian AY, Eichenfield LF, Diaz LZ, Horev A et al Hidradenitis Suppurativa in the Pediatric Population. An International, Retrospective, Cross-sectional Study of 481 Pediatric Patients. JAMA Dermatol 2021;157(4):385-391 doi:10.1001/jamadermatol.2020.5435
6) Jenei A, Dajnoki Z, Medgyesi B, Gáspár K, Béke G, Kinyó Á, Méhes G, Hendrik Z, Dinya T, Törőcsik D, Zouboulis CC, Prens EP, Bíró T, Szegedi A, Kapitány A. Apocrine Gland-Rich Skin Has a Non-Inflammatory IL-17-Related Immune Milieu, that Turns to Inflammatory IL-17-Mediated Disease in Hidradenitis Suppurativa. J Invest Dermatol. 2019 Apr;139(4):964-968. doi: 10.1016/j.jid.2018.10.020.
7) Naasan H, Affleck A. Atypical hidradenitis suppurativa. Clin Exp Dermatol. 2015 Dec;40(8):891-3. doi: 10.1111/ced.12655.
8) van der Zee HH, Jemec GB. New insights into the diagnosis of hidradenitis suppurativa: Clinical presentations and phenotypes. J Am Acad Dermatol. 2015 Nov;73(5 Suppl 1):S23-6. doi: 10.1016/j.jaad.2015.07.047.

9) Lipsker D, Severac F, Freysz M, Sauleau E, Boer J, EMtestam L et al The ABC of Hidradenitis Suppurativa: A Validated Glossary on how to Neame Lesions. Dermatology 2016;232:137-142 doi.org/10.1159/000443878
10) Frew JW, Lowes MA, Goldfarb N, Butt M, Piguet V, O'Brien E, Ingram J, Jemec GBE, Tan J, Zouboulis C, Alavi A, Kirby JS. Global Harmonization of Morphological Definitions in Hidradenitis Suppurativa for a Proposed Glossary. JAMA Dermatol. 2021 Apr 1;157(4):449-455. doi: 10.1001/jamadermatol.2020.5467.
11) Navrazhina K, Frew JW, Gilleaudeau P, Sullivan-Whalen M, Garcet S, Krueger JG. Epithelialized tunnels are a source of inflammation in hidradenitis suppurativa. J Allergy Clin Immunol. 2021 Jun;147(6):2213-2224. doi: 10.1016/j.jaci.2020.12.651.
12) Phan, K., Charlton, O. & Smith, S.D. Global prevalence of hidradenitis suppurativa and geographical variation—systematic review and meta-analysis. biomed dermatol 4, 2 (2020). https://doi.org/10.1186/s41702-019-0052-0
13) Saunte DM, Boer J, Stratigos A, Szepietowski JC, Hamzavi I, Kim KH, Zarchi K, Antoniou C, Matusiak L, Lim HW, Williams M, Kwon HH, Gürer MA, Mammadova F, Kaminsky A, Prens E, van der Zee HH, Bettoli V, Zauli S, Hafner J, Lauchli S, French LE, Riad H, El-Domyati M, Abdel-Wahab H, Kirby B, Kelly G, Calderon P, del Marmol V, Benhadou F, Revuz J, Zouboulis CC, Karagiannidis I, Sartorius K, Hagströmer L, McMeniman E, Ong N, Dolenc-Voljc M, Mokos ZB, Borradori L, Hunger RE, Sladden C, Scheinfeld N, Moftah N, Emtestam L, Lapins J, Doss N, Kurokawa I, Jemec GB. Diagnostic delay in hidradenitis suppurativa is a global problem. Br J Dermatol. 2015 Dec;173(6):1546-9. doi: 10.1111/bjd.14038.
14) Naik HB, Paul M, Cohen SR, Alavi A, Suàrez-Fariñas M, Lowes MA. Distribution of Self-reported Hidradenitis Suppurativa Age at Onset. JAMA Dermatol. 2019;155(8):971–973. doi:10.1001/jamadermatol.2019.0478
15) Kamil MAA, Chandran NS, Sulistyaningrum SK, Rajagopalan M, Tahiliani S, Hsiao J, Naik H, Shi V, Frew JW. The Asia Pacific Hidradenitis Suppurativa

(APHiS) foundation: promoting hidradenitis suppurativa research in the Asia Pacific. Int J Dermatol. 2020 Aug;59(8):e289-e290. doi: 10.1111/ijd.14960.

16) Deckers IE, Janse IC, van der Zee HH, Nijsten T, Boer J, Horváth B, Prens EP. Hidradenitis suppurativa (HS) is associated with low socioeconomic status (SES): A cross-sectional reference study. J Am Acad Dermatol. 2016 Oct;75(4):755-759.e1. doi: 10.1016/j.jaad.2016.04.067.

17) Choi E, Cook AR, Chandran NS. Hidradenitis Suppurativa: An Asian Perspective from a Singaporean Institute. Skin Appendage Disord. 2018 Oct;4(4):281-285. doi: 10.1159/000481836.

18) Zouboulis CC, Del Marmol V, Mrowietz U, Prens EP, Tzellos T, Jemec GB. Hidradenitis Suppurativa/Acne Inversa: Criteria for Diagnosis, Severity Assessment, Classification and Disease Evaluation. Dermatology. 2015;231(2):184-90. doi: 10.1159/000431175.

19) Frew JW, Marzano AV, Wolk K, Join-Lambert O, Alavi A, Lowes MA, Piguet V. A Systematic Review of Promising Therapeutic Targets in Hidradenitis Suppurativa: A Critical Evaluation of Mechanistic and Clinical Relevance. J Invest Dermatol. 2021 Feb;141(2):316-324.e2. doi: 10.1016/j.jid.2020.06.019.

20) Wolk K, Wenzel J, Tsaousi A, Witte-Händel E, Babel N, Zelenak C, Volk HD, Sterry W, Schneider-Burrus S, Sabat R. Lipocalin-2 is expressed by activated granulocytes and keratinocytes in affected skin and reflects disease activity in acne inversa/hidradenitis suppurativa. Br J Dermatol. 2017 Nov;177(5):1385-1393. doi: 10.1111/bjd.15424.

21) van der Zee HH, de Ruiter L, van den Broecke DG, Dik WA, Laman JD, Prens EP. Elevated levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-α and IL-1β. Br J Dermatol. 2011 Jun;164(6):1292-8. doi: 10.1111/j.1365-2133.2011.10254.x.

22) Matusiak Ł, Bieniek A, Szepietowski JC. Hidradenitis suppurativa markedly decreases quality of life and professional activity. J Am Acad Dermatol. 2010 Apr;62(4):706-8, 708.e1. doi: 10.1016/j.jaad.2009.09.021.

23) Matusiak L Profound consequences of hidradenitis suppurativa: a review. Br J Dermatol 2020; 183(6): e171-e177

24) Tzellos T, Yang H, Mu F, Calimlim B, Signorovitch J. Impact of hidradenitis suppurativa on work loss, indirect costs and income. Br J Dermatol. 2019 Jul;181(1):147-154. doi: 10.1111/bjd.17101.
25) Krisztián Gáspár, L. Hunor Gergely, Balázs Jenei, Norbert Wikonkál, Ágnes Kinyó, Andrea Szegedi, Éva Remenyik, Norbert Kiss, Xiang Jin, Miklós Sárdy, Zsuzsanna Beretzky, Márta Péntek, László Gulácsi, András Bánvölgyi, Valentin Brodszky & Fanni Rencz (2021) Resource utilization, work productivity and costs in patients with hidradenitis suppurativa: a cost-of-illness study, Expert Review of Pharmacoeconomics & Outcomes
Research, DOI: 10.1080/14737167.2021.1895753
26) Frew JW, Krueger JG What's in the pipeline for Hidradenitis Suppurativa? Emerging and Novel Therapeutics. Drugs Fut 2021 46(1): 43
27) Alikhan A, Sayed C, Alavi A, Alhusayen R, Brassard A, Burkhart C, Crowell K, Eisen DB, Gottlieb AB, Hamzavi I, Hazen PG, Jaleel T, Kimball AB, Kirby J, Lowes MA, Micheletti R, Miller A, Naik HB, Orgill D, Poulin Y. North American clinical management guidelines for hidradenitis suppurativa: A publication from the United States and Canadian Hidradenitis Suppurativa Foundations: Part I: Diagnosis, evaluation, and the use of complementary and procedural management. J Am Acad Dermatol. 2019 Jul;81(1):76-90. doi: 10.1016/j.jaad.2019.02.067.
28) Alikhan A, Sayed C, Alavi A, Alhusayen R, Brassard A, Burkhart C, Crowell K, Eisen DB, Gottlieb AB, Hamzavi I, Hazen PG, Jaleel T, Kimball AB, Kirby J, Lowes MA, Micheletti R, Miller A, Naik HB, Orgill D, Poulin Y. North American clinical management guidelines for hidradenitis suppurativa: A publication from the United States and Canadian Hidradenitis Suppurativa Foundations: Part II: Topical, intralesional, and systemic medical management. J Am Acad Dermatol. 2019 Jul;81(1):91-101. doi: 10.1016/j.jaad.2019.02.068
29) Ingram JR, Collier F, Brown D, Burton T, Burton J, Chin MF, Desai N, Goodacre TEE, Piguet V, Pink AE, Exton LS, Mohd Mustapa MF. British Association of Dermatologists guidelines for the management of hidradenitis suppurativa (acne

inversa) 2018. Br J Dermatol. 2019 May;180(5):1009-1017. doi: 10.1111/bjd.17537.

30) Zouboulis CC, Desai N, Emtestam L, Hunger RE, Ioannides D, Juhász I, Lapins J, Matusiak L, Prens EP, Revuz J, Schneider-Burrus S, Szepietowski JC, van der Zee HH, Jemec GB. European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. J Eur Acad Dermatol Venereol. 2015 Apr;29(4):619-44. doi: 10.1111/jdv.12966.

31) Riddle A, Westerkam L, Feltner C, Sayed C. Current Surgical Management of Hidradenitis Suppurativa: A Systematic Review and Meta-Analysis. Dermatol Surg. 2021 Jan 11. doi: 10.1097/DSS.0000000000002892.

32) Goldburg SR, Strober BE, Payette MJ. Hidradenitis suppurativa: Current and emerging treatments. J Am Acad Dermatol. 2020 May;82(5):1061-1082. doi: 10.1016/j.jaad.2019.08.089.

33) Frings VG, Bauer B, Glöditzsch M, Goebeler M, Presser D. Assessing the psychological burden of patients with hidradenitis suppurativa. Eur J Dermatol. 2019 Jun 1;29(3):294-301. doi: 10.1684/ejd.2019.3552.

34) Cuenca-Barrales C, Ruiz-Villaverde R, Molina-Leyva A. Sexual Distress in Patients with Hidradenitis Suppurativa: A Cross-Sectional Study. J Clin Med. 2019;8(4):532. Published 2019 Apr 18. doi:10.3390/jcm8040532

35) Hoffman LK, Ghias MH, Garg A, Hamzavi IH, Alavi A, Lowes MA. Major gaps in understanding and treatment of hidradenitis suppurativa. Semin Cutan Med Surg. 2017 Jun;36(2):86-92. doi: 10.12788/j.sder.2017.024.

36) Frew JW, Vekic DA, Woods J, Cains GD. Phenotypic heterogeneity implies heterogeneous pathogenic pathways in hidradenitis suppurativa. Exp Dermatol. 2015 May;24(5):338-9. doi: 10.1111/exd.12648.

37) Theut Riis P, Saunte DM, Benhadou F, Del Marmol V, Guillem P, El-Domyati M, Abdel-Wahab H, Antoniou C, Dessinioti C, Gürer MA, Beksaç B, Szepietowski JC, Matusiak L, Emtestam L, Lapins J, Riad H, Doss N, Massa AF, Hamzavi I, Nicholson C, Dolenc-Voljc M, Kim KH, Ohn J, Zouboulis CC, Karagiannidis I, Mokos ZB, Durinec P, Jemec GBE. Low and high body mass index in

hidradenitis suppurativa patients-different subtypes? J Eur Acad Dermatol Venereol. 2018 Feb;32(2):307-312. doi: 10.1111/jdv.14599.

38) González-Manso A, Agut-Busquet E, Romaní J, Vilarrasa E, Bittencourt F, Mensa A, Cantó E, Aróstegui JI, Vidal S. Hidradenitis Suppurativa: Proposal of Classification in Two Endotypes with Two-Step Cluster Analysis. Dermatology. 2021;237(3):365-371. doi: 10.1159/000511045.

39) Allen L, O'Connell A, Kiermer V How can we ensure visibility and diversity in research contributions? How the Contributor Role Taxonomy (CRediT) is helping the shift from authorship to contributorship Learn Publish. 2019;32(1):71-74

40) Shelley WB, Cahn MM. The pathogenesis of hidradenitis suppurativa in man; experimental and histologic observations. AMA Arch Derm. 1955 Dec;72(6):562-5.

41) Jemec GB, Hansen U. Histology of hidradenitis suppurativa. J Am Acad Dermatol. 1996 Jun;34(6):994-9.

42) Melnik BC, Plewig G. Impaired Notch signalling: the unifying mechanism explaining the pathogenesis of hidradenitis suppurativa (acne inversa). Br J Dermatol. 2013 Apr;168(4):876-8.

43) Melnik BC, Plewig G Impaired Notch-MKP-1 signalling in hidradenitis suppurativa: an approach to pathogenesis by evidence from translational biology Exp Dermatol 2013;22(3):172-177

44) Frings VG, Sennefelder H, Presser D, Goebeler M, Schmidt M. Altered NOX expression does not seem to account for epidermal NLRP3 inflammasome activation in hidradenitis suppurativa. Br J Dermatol. 2019 Aug;181(2):391-392.

45) Shah A, Alhusayen R, Amini-Nik S. The critical role of macrophages in the pathogenesis of hidradenitis suppurativa. Inflamm Res. 2017 Nov;66(11):931-945.

46) Lima AL, Karl I, Giner T, Poppe H, Schmidt M, Presser D, Goebeler M, Bauer B. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. Br J Dermatol. 2016 Mar;174(3):514-21.

47) Marzano AV, Borghi A, Meroni PL, Cugno M. Pyoderma gangrenosum and its syndromic forms: evidence for a link with autoinflammation. Br J Dermatol. 2016 Nov;175(5):882-891.
48) Ghias MH, Hyde MJ, Tomalin LE, Morgan BP, Alavi A, Lowes MA, Piguet V. Role of the Complement Pathway in Inflammatory Skin Diseases: A Focus on Hidradenitis Suppurativa. J Invest Dermatol. 2020 Mar;140(3):531-536.e1.
49) Kanni T, Zenker O, Habel M, Riedemann N, Giamarellos-Bourboulis EJ. Complement activation in hidradenitis suppurativa: a new pathway of pathogenesis? Br J Dermatol. 2018 Aug;179(2):413-419.
50) Witte-Handel E, Wolk K, Tsausi A, Irmer ML, Mossner R, Shomroni O, et al The IL-1 Pathway is Hyperactive in Hidradenitis Suppurativa and Contributed to Skin Infiltration and Destruction J Invest Dermatol 2019;139(6):1294-1305
51) Wolk K, Warszawska K, Hoeflich C, Witte E, Schneider-Burus S, Witte K et al Deficiency of IL-22 contribute to a chronic inflammatory disease: pathogenetic mechanisms in acne inversa J Immunol 2011;186(2):1228-39
52) Tzanetakou V, Kanni T, Giatrakou S, Katoulid A, Papadavid E, Netea MG JAMA Dermatol 2016;152(1):52-59
53) Gottlieb A, Natsis NE, Kerdel F, Forman S, Gonzalez E, Jimenez G, Hernandez L et al A Phase II Open-Label Study og Bermekimab in Patients with Hidradenitis Suppurativa Shows Resolution of Inflammatory Lesions and Pain J Invest Dermatol 2020; doi:10.1016/j.joid.2019.20.024
54) Nomura T Hidradenitis Suppurativa as a Potnetial Subtype of Autoinflammatory Keratinization Disease Front. Immunol. 11:847. doi: 10.3389/fimmu.2020.00847
55) Jimenez-Gallo D, de la Varga-Martinez R, Ossorio-Garcia L, Albarran-PLanelles C, Rodriguez C, Linares-Barrios M. The Clinical Significance of Increased Serum Proinflammatory Cytokines, C-Reactive Protein, and Erythrocyte Sedimentation Rate in Patients with Hidradenitis Suppurativa. Mediators Inflamm 2017;2450401.doi: 10.1155/2017/2450401
56) Kanni T, Tzanetakou V, Savva A, Kersten B, PIstiki A, van de Veerdonk FL et al Compartmentalized Cytokine Responses in Hidradenitis Suppurativa PLoS One. 2015;10(6):e0130522

57) Byrd AS, Carmona-Rivera C, O'Neil LJ, Carlucci PM, Cisar C, Rosenberg AZ, Kerns ML, Caffrey JA, Milner SM, Sacks JM, Aliu O, Broderick KP, Reichner JS, Miller LS, Kang S, Robinson WH, Okoye GA, Kaplan MJ. Neutrophil extracellular traps, B cells, and type I interferons contribute to immune dysregulation in hidradenitis suppurativa. Sci Transl Med. 2019 Sep 4;11(508):eaav5908. doi: 10.1126/scitranslmed.aav5908.

58) Lowe MM, Naik HB, Clancy S, Pauli M, Smith KM, Bi Y, Dunstan R, Gudjonsson JE, Paul M, Harris H, Kim E, Shin US, Ahn R, Liao W, Hansen SL, Rosenblum MD. Immunopathogenesis of hidradenitis suppurativa and response to anti-TNF-α therapy. JCI Insight. 2020 Oct 2;5(19):e139932. doi: 10.1172/jci.insight.139932.

59) Musilova J, Moran B, Sweeney CM, Malara A, Zaborowski A, Hughes R, Winter DC, Fletcher JM, Kirby B. Enrichment of Plasma Cells in the Peripheral Blood and Skin of Patients with Hidradenitis Suppurativa. J Invest Dermatol. 2019 Nov 1. pii: S0022-202X(19)33374-3. doi: 10.1016/j.jid.2019.08.453.

60) Hoffman LK, Tomalin LE, Schultz G, Howell MD, Anandasabapathy N, Alavi A, Suárez-Fariñas M, Lowes MA. Integrating the skin and blood transcriptomes and serum proteome in hidradenitis suppurativa reveals complement dysregulation and a plasma cell signature. PLoS One. 2018 Sep 28;13(9):e0203672. doi: 10.1371/journal.pone.0203672. eCollection 2018.

61) Vossen ARJV, van der Zee HH and Prens EP (2018) Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model. Front. Immunol. 9:2965.

62) Krnic Martinic, M., Pieper, D., Glatt, A. et al. Definition of a systematic review used in overviews of systematic reviews, meta-epidemiological studies and textbooks. BMC Med Res Methodol 19, 203 (2019). https://doi.org/10.1186/s12874-019-0855-0

63) Michelle A. Lowes, Mayte Suárez-Fariñas, and James G. Krueger Immunology of Psoriasis Annual Review of Immunology 2014 32:1, 227-255 doi: 10.1146/annurev-immunol-032713-120225

64) Honda T, Kabashima K. Reconciling innate and acquired immunity in atopic dermatitis. J Allergy Clin Immunol. 2020 Apr;145(4):1136-1137. doi: 10.1016/j.jaci.2020.02.008OI:https://doi.org/10.1016/j.jaci.2020.02.008
65) Von von Laffert M, Stadie V, Wohlrab J, Marsch WC. Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae. Br J Dermatol. 2011 Feb;164(2):367-71
66) von Laffert M[1], Helmbold P, Wohlrab J, Fiedler E, Stadie V, Marsch WC. Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. Exp Dermatol. 2010 Jun;19(6):533-7.
67) Danby FW, Jemec GB, Marsch WCh, von Laffert M. Preliminary findings suggest hidradenitis suppurativa may be due to defective follicular support. Br J Dermatol. 2013 May;168(5):1034-9.
68) Fitzsimmons JS, Fitzsimmons EM and Guilbert PR (1984) Familial hidradenitis suppurativa: evidence in favour of single gene transmission. J Med Genet 21:281–5
69) Fitzsimmons JS, Guilbert PR and Fitzsimmons EM (1985) Evidence of genetic factors in hidradenitis suppurativa. Br J Dermatol 113:1–8
70) Wang B, Yang W, Wen W, Sun J, Su B, Liu B et al Gamma-secretase gene mutations in familial acne inversa Science 2010;330(6007):1065
71) Jfri AH, O'Brien EA, Litvinov IV, Alavi A, Netchiporouk E. Hidradenitis Suppurativa: Comprehensive Review of Predisposing Genetic Mutations and Changes. J Cutan Med Surg. 2019 Sep/Oct;23(5):519-527. doi: 10.1177/1203475419852049.
72) Xiao X, He Y, Li C, Zhang X, Xu H, Wang B. Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signalling pathways. Br J Dermatol. 2016 Mar;174(3):522-32. doi: 10.1111/bjd.14223.
73) Duchatelet S, Miskinyte S, Delage M, Ungeheuer MN, Lam T, Benhadou F, Del Marmol V, Vossen ARJV, Prens EP, Cogrel O, Beylot-Barry M, Girard C, Vidil J, Join-Lambert O, Parisot M, Nitschké P, Hanein S, Fraitag S, Van der Zee HH, Bessis D, Damiani G, Altomare A, Liao YH, Nikolakis G, Zouboulis CC, Nassif A,

Hovnanian A. Low Prevalence of GSC Gene Mutations in a Large Cohort of Predominantly Caucasian Patients with Hidradenitis Suppurativa. J Invest Dermatol. 2020 Oct;140(10):2085-2088.e14. doi: 10.1016/j.jid.2019.10.025.

74) Hessam, S., Gambichler, T., Skrygan, M., Scholl, L., Sand, M., Meyer, T., ... & Bechara, F. G. (2021). Increased expression profile of NCSTN, Notch and PI3K/AKT3 in hidradenitis suppurativa. Journal of the European Academy of Dermatology and Venereology, 35(1), 203-210.

75) Frew JW. Differential profiles of gamma secretase Notch signalling in hidradenitis suppurativa: the need for genotype-endotype-phenotype analysis. Br J Dermatol. 2021 Jan 9. doi: 10.1111/bjd.19805.

76) Kimball AB High placebo rates in clnical trials: is the problem scoring systems or drug efficacy? J Am Acad Dermatol 83(6):e431

77) Byrd, A.S., Dina, Y., Okoh, U.J. et al. Specimen Collection for Translational Studies in Hidradenitis Suppurativa. Sci Rep 9, 12207 (2019). https://doi.org/10.1038/s41598-019-48226-w

78) Amir Ali A, Seng EK, Alavi A, Lowes MA. Exploring changes in placebo treatment arms in hidradenitis suppurativa randomized clinical trials: A systematic review. J Am Acad Dermatol. 2020 Jan;82(1):45-53. doi: 10.1016/j.jaad.2019.05.065.

79) Zouboulis CC, Tzellos T, Kyrgidis A, Jemec GBE, Bechara FG, Giamarellos-Bourboulis EJ, Ingram JR, Kanni T, Karagiannidis I, Martorell A, Matusiak Ł, Pinter A, Prens EP, Presser D, Schneider-Burrus S, von Stebut E, Szepietowski JC, van der Zee HH, Wilden SM, Sabat R; European Hidradenitis Suppurativa Foundation Investigator Group. Development and validation of the International Hidradenitis Suppurativa Severity Score System (IHS4), a novel dynamic scoring system to assess HS severity. Br J Dermatol. 2017 Nov;177(5):1401-1409. doi: 10.1111/bjd.15748.

80) Goldfarb N, Ingram JR, Jemec GBE, Naik HB, Piguet V, Hyde MJ, Freese R, Lowes MA, Alavi A. Hidradenitis Suppurativa Area and Severity Index (HASI): a pilot study to develop a novel instrument to measure the physical signs of hidradenitis suppurativa. Br J Dermatol. 2020 Jan;182(1):240-242. doi: 10.1111/bjd.18335.

81) Hawkes JE, Chan TC, Krueger JG. Psoriasis pathogenesis and the development of novel targeted immune therapies. J Allergy Clin Immunol. 2017 Sep;140(3):645-653. doi: 10.1016/j.jaci.2017.07.004.

82) Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. Br J Dermatol. 2020 Apr;182(4):1045-1047. doi: 10.1111/bjd.18556.

83) Retrouvey H, Webster F, Zhong T, et al Cross-sectional analysis of bibliometrics and altmetrics: comparing the impact of qualitative and quantitative articles in the British Medical Journal BMJ Open 2020;10:e040950. doi: 10.1136/bmjopen-2020-040950

84) National Library of Medicine Safety and Efficacy of Avacopan in Subjects with Moderate to Severe Hidradenitis Suppurativa. Clinicaltrials.gov Identifier NCT03852472 https://clinicaltrials.gov/ct2/show/NCT03852472 Retrieved 3rd July 2021

85) National Library of Medicine. Efficacy and Safety Study of IFX-1 in Patients with Moderate to Severe Hidradenitis Suppurativa. Clinical Trials.gov Identifier NCT03487276 https://clinicaltrials.gov/ct2/show/NCT03487276 Retrieved 3rd July 2021

86) Gudjonsson JE, Tsoi LC, Ma F, et al. Contribution of plasma cells and B cells to hidradenitis suppurativa pathogenesis. *JCI Insight*. 2020;5(19):e139930. Published 2020 Oct 2. doi:10.1172/jci.insight.139930

APPENDICES:

Appendix A CRediT Definitions and Author Contributions:

- **Conceptualization** – Ideas; formulation or evolution of overarching research goals and aims.
- **Data curation** – Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.
- **Formal analysis** – Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.
- **Funding acquisition** - Acquisition of the financial support for the project leading to this publication.
- **Investigation** – Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.
- **Methodology** – Development or design of methodology; creation of models.
- **Project administration** – Management and coordination responsibility for the research activity planning and execution.
- **Resources** – Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools.
- **Software** – Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.
- **Supervision** – Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.
- **Validation** – Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.
- **Visualization** – Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.
- **Writing – original draft** – Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).
- **Writing – review & editing** – Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre- or post-publication stages.

Author Contributions:

Publication 1-1: Frew JW, Hawkes JE, Krueger JG "A Systematic Review and Critical Evaluation of Inflammatory Cytokine Associations in Hidradenitis Suppurativa" *F1000Research* (2018); 2018 Dec 13;7:1930

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; JEH: Data Curation; Investigation; Formal Analysis Writing- Reviewing and Editing; JGK: Writing- Reviewing and Editing

Publication 1-2: Frew JW, Hawkes JE, Krueger JG "A Systematic Review and Critical Evaluation of Immunohistochemical Associations in Hidradenitis Suppurativa" *F1000Research* (2018); 2018, 7:1923

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; JEH: Data Curation; Investigation; Formal Analysis Writing- Reviewing and Editing; JGK: Writing- Reviewing and Editing

Publication 1-3: Frew JW, Hawkes JE, Krueger JG "Topical, Systemic and Biologic Therapies in Hidradenitis Suppurativa: Pathogenic Insights Through Examination of Therapeutic Mechanisms" (2018) *Therapeutic Advances in Chronic Disease* 2019; 10: 2040622319830646

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; JEH: Data Curation; Investigation; Formal Analysis Writing- Reviewing and Editing; JGK: Writing- Reviewing and Editing

Publication 2-1: Frew JW, Navrazhina K, Maroun M, Lu PJ, Krueger JG Contribution of Fibroblasts to Tunnel Formation and Inflammation in Hidradenitis Suppurativa Exp Dermatol 2019 28(8):886-891

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; KN: Formal Analysis, Writing- Reviewing and Editing; MM: Writing- Reviewing and Editing; PJL: Writing- Reviewing and Editing; JGK: Writing- Reviewing and Editing.

Publication 2-2: Grand D, Navrazhina K, Frew JW Integrating Complement into the Molecular Pathogenesis of Hidradenitis Suppurativa Exp Dermatol 2020; 29(1): 86-92

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; DG: Formal Analysis, Writing- Reviewing and Editing; KN: Formal Analysis, Writing- Reviewing and Editing

Publication 2-3: Frew JW, Grand D, Navrazhina K, Krueger JG "Beyond Antibodies: B-cells in Hidradenitis Suppurativa: Bystanders, Contributors or Therapeutic Targets?" Exp Dermatol 2020; 29(5):509-515

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; DG: Formal Analysis, Writing- Reviewing and Editing; KN: Formal Analysis, Writing-Reviewing and Editing

Publication 2-4: Frew JW. Hidradenitis Suppurativa is an Autoinflammatory Keratinization Disease: A Review of the Clinical, Histological and Molecular Evidence: JAAD International 2020;1(1):62-72

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 2-S1: Navrazhina K*, Frew JW*, Garcet S, Krueger JG. Epithelialized Tunnels Contribute to Inflammation in Hidradenitis Suppurativa. J Allergy Clin Immunol 2020; doi:10.1016/j.jaci.2020.12.651

CRediT Contribution: JF: Data Curation; Investigation; Visualisation; Writing- Reviewing and Editing; KN: Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; SG: Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 2-S2: Navrazhina K, Garcet S, Gonzales J, Grand D, Frew JW, Krueger JG In-Depth Analysis of the Hidradenitis Suppurativa Serum Proteome Identified Distinct Inflammatory Subtypes. J Invest Dermatol 2021; doi:10.1016/j.jid.2021.02.742

CRediT Contribution: JF: Data Curation; Investigation; Writing- Reviewing and Editing; KN: Data Curation; Investigation; Formal Analysis; Visualisation; JG: Data Curation; Investigation; Formal Analysis; Visualisation; DG: Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; SG: Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 3-1: Frew JW, Vekic DA, Woods J, Cains GD (2017) "A Systematic Review and Critical Evaluation of Reported Pathogenic Sequence Variants in Hidradenitis Suppurativa" British Journal of Dermatology 2017; DOI:10.1111/bjd.15441

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; DV: Formal Analysis, Writing- Reviewing and Editing; JW: Writing- Reviewing and Editing; GC: Writing- Reviewing and Editing

Publication 3-2: Frew JW, Hawkes JE, Sullivan-Whalen M, Gilleaudeau P, Krueger JG "Inter-Relater Reliability of Phenotypes, and Exploratory Genotype- Phenotype Analysis in Inherited Hidradenitis Suppurativa" Br J Dermatol 2019; 2019 Jan 28. doi: 10.1111/bjd.1769

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing. JEH: Formal Analysis; Visualisation, Writing- Reviewing and Editing; MS: Writing- Reviewing and Editing; PG: Writing- Reviewing and Editing; JGK: Writing- Reviewing and Editing

Publication 3-3: Frew JW, Navrazhina K, In-Silico Analysis of Gamma-Secretase-Complex Mutations in Hidradenitis Suppurativa Demonstrates Disease-Specific Substrate Recognition and Cleavage Alterations Front Med 2019; DOI: 10.3389/fmed.2019.00206

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing KN: Investigation; Formal Analysis, Writing- Reviewing and Editing

Publication 3-4: Frew JW "We Need to Talk About Notch: Notch Dysregulation as an Epiphenomenon in Inflammatory Skin Disease" (2018) Br J Dermatol 2019 Feb;180(2):431-432

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 3-5: Frew JW Navrazhina K No Evidence that Impaired Notch Signalling Differentiates Hidradenitis Suppurativa from other Inflammatory Skin Diseases Br J Dermatol 2020;182(4):1042-1043

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; KN: Investigation; Formal Analysis, Writing- Reviewing and Editing

Publication 4-1: Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger JG Dermal Tunnels Influence Time to Clinical Response and Family History Influences Time to Loss of Clinical Response in Hidradenitis Suppurativa Patients Treated with Adalimumab. Clin Exp Dermatol 2020; 46(2):306-313

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; CJ: Data Curation, Formal Analysis; NS: Data Curation, Formal Analysis; RV: Data Curation, Formal Analysis; DG: Data Curation, Formal Analysis; KN: Data Curation, Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 4-2: Frew JW, Singh N, Jiang CS, Navrazhina K, Vaughan R, Krueger JG. The Impact of Body Mass Index Upon the Efficacy of Adalimumab in Hidradenitis Suppurativa. Front Medicine 2021;8:603281

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; CJ: Data Curation, Formal Analysis; NS: Data Curation, Formal Analysis; RV: Data Curation, Formal Analysis; DG: Data Curation, Formal Analysis; KN: Data Curation, Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 4-3: Frew JW, Jiang C, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger J "Malignancy and Infection Risk During Adalimumab Therapy in Hidradenitis Suppurativa" Clin Exp Dermatol 2020; 45(7):859-865

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; CJ: Data Curation, Formal Analysis; NS: Data Curation, Formal Analysis; RV: Data Curation, Formal Analysis; DG: Data Curation, Formal Analysis; KN: Data Curation, Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 5-1: Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR et al Defining Lesional, Perilesional and Unaffected Skin in Hidradenitis Suppurativa: Proposed Recommendations for Clinical Trials and Translational Research Studies Br J Dermatol 2019; 181(6):1339-1341

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; KN: Formal Analysis, Writing- Reviewing and Editing; AB: Formal Analysis, Writing- Reviewing and Editing; AG: Formal Analysis, Writing- Reviewing and Editing; JI: Formal Analysis, Writing- Reviewing and Editing

Publication 5-2: Frew JW, Jiang C, Singh N, Grand D, Navrazhina K Vaughan R, Krueger JG Clinical Response Rates, Placebo Response Rates and Significantly Associated Covariates Are Dependent Upon Choice of Outcome Measure in Hidradenitis Suppurativa: A Post-Hoc Analysis of PIONEER 1 and 2 Individual Patient Data J Am Acad Dermatol 2019; 82(5):1150-1157

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; CJ: Data Curation, Formal Analysis; NS: Data Curation, Formal Analysis; RV: Data Curation,

Formal Analysis; DG: Data Curation, Formal Analysis; KN: Data Curation, Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 5-3: Frew JW "Primary Imputation Methods Impact Efficacy Results in Hidradenitis Suppurativa Clinical Trials" J Am Acad Dermatol 2020; 83(2):663-665

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing

Publication 5-4: Frew JW, Jiang CS, Singh N, Navrazhina K, Vaughan R, Krueger JG Quantifying the Natural Variation in Lesion Counts over time in Untreated Hidradenitis Suppurativa: Implications for Outcome Measures and Trial Design. JAAD International 2020; 1(2):208-221

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; CJ: Data Curation, Formal Analysis; NS: Data Curation, Formal Analysis; RV: Data Curation, Formal Analysis; DG: Data Curation, Formal Analysis; KN: Data Curation, Formal Analysis; JGK: Writing- Reviewing and Editing

Publication 6-1: Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, Ungar J, Krueger JG The Effect of Subcutaneous Brodalumab upon Clinical Disease Activity in Hidradenitis Suppurativa: An Open Label Cohort Study. J Am Acad Dermatol 2020; 83(5):1341-1348

CRediT Contribution: JF: Conceptualisation; Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing. DG:

Formal Analysis; Visualisation, Writing- Reviewing and Editing; KN: Formal Analysis; Visualisation, Writing- Reviewing and Editing; MS: Writing- Reviewing and Editing; PG: Writing- Reviewing and Editing; JU: Formal Analysis; Visualisation, Writing- Reviewing and Editing; JGK: Writing- Reviewing and Editing

Publication 6-2: Frew JW, Navrazahina K, Garcet S, Sullivan-Whalen M, Gilleaudeau P, Krueger JG Weekly Administration of Brodalumab in Hidradenitis Suppurativa: An Open Label Cohort Study. Br J Dermatol 2020; 184(2):350-352

CRediT Contribution: JF: Data Curation; Investigation; Formal Analysis; Visualisation; Writing-Original Draft; Writing- Reviewing and Editing; KN: Formal Analysis; Visualisation, Writing- Reviewing and Editing; SGH: Formal Analysis MS: Writing-Reviewing and Editing; PG: Writing- Reviewing and Editing; JGK: Writing-Reviewing and Editing

Appendix B: List of all Publications by the Author

Peer Reviewed Publications:

1) Flora A, Smith A, Holland R, Frew JW Rapid and Sustained Remission of SAPHO with IL-23p19 Antagonism. JAAD Case Reports 2021; 14(3): doi: 10.1016/j.jdcr.2021.05.029
2) Navrazhina K, Garcet S, Zheng X, Hur HB, Frew JW, Krueger JG. High inflammation in HS extends to perilesional skin, and can be subdivided by LCN2 expression J Allerg Clin Immunol 2021: doi: https://doi.org/10.1016/j.jaci.2021.05.027
3) Sarkissian S, Frew JW Ultrasound-Guided De-Roofing of Epithelialised Tunnels of Hidradenitis Suppurativa. Aust J Dermatol 2021; 62(3):360-363
4) Frew JW, Singh N, Jiang CS, Navrazhina K, Vaughan R, Krueger JG. The Impact of Body Mass Index Upon the Efficacy of Adalimumab in Hidradenitis Suppurativa. Front Medicine 2021; 8; 603281
5) Sarkissian S, Cachia A, Frew JW Cellular Neurothekeoma. Int J Womens Dermatol 2021; doi:10.1016/j.ijwd.2021.07.011
6) Mintoff D, Banhadou F, Pace N, Frew JW Metabolic Syndrome and Hidradenitis Suppurativa; Epidemiological, Molecular and Therapeutic Aspects. Int J Dermatol 2021;doi:10.1111/ijd.15910
7) Frew JW, Grand D, Navrazhina K, Garcet S, Krueger JG Assessing the Responsiveness of Sonographic Biomarkers to Brodalumab Therapy in Hidradenitis Suppurativa. J Eur Acad Dermatol Venereol 2021;35(12): e884-e887
8) Frew JW, Lowes MA, Goldfarb N, Butt M, Piguet V, O'Brien R, Ingram J, Jemec GBE, Tan J, Zouboulis CC, Alavi A, Kirby JS Global Harmonization of Morphological Definitions in Hidradenitis Suppurativa for a proposed HS Glossary. JAMA Dermatol 2021; doi:10.1001/jamadermatol.2020.5467
9) Frew JW Differential Profiles of Gamma-Secretase-Notch Signaling in Hidradenitis Suppurativa: The need for Genotype-Endotype-Phenotype Analysis. Br J Dermatol 2021; doi:10.1111/bjd.19805

10) Navrazhina K, Garcet S, Gonzales J, Grand D, Frew JW, Krueger JG In-Depth Analysis of the Hidradenitis Suppurativa Serum Proteome Identified Distinct Inflammatory Subtypes. J Invest Dermatol 2021; doi:10.1016/j.jid.2021.02.742
11) Michael W Tee, Andrew B Avarbock, Jonathan Ungar, John W Frew Rapid resolution of Pyoderma Gangrenosum with Brodalumab Therapy. JAAD Case Reports 2020; 6(11):1167-1169
12) Zouboulis CC, Benhadou F et al What causes Hidradenitis Suppurativa? – 15 years after. Exp Dermatol 2020; 29(12):1154-1170
13) Frew JW, Krueger JG What's in the pipeline for Hidradenitis Suppurativa Drugs of the Future- Clarivate Analytics 2021; 46(1):43
14) Frew JW, Jiang CS, Singh N, Navrazhina K, Vaughan R, Krueger JG Quantifying the Natural Variation in Lesion Counts over time in Untreated Hidradenitis Suppurativa: Implications for Outcome Measures and Trial Design. JAAD International 2020; 1(2):208-221
15) Frew JW, Navrazahina K, Garcet S, Sullivan-Whalen M, Gilleaudeau P, Krueger JG Weekly Administration of Brodalumab in Hidradenitis Suppurativa: An Open Label Cohort Study. Br J Dermatol 2020; 184(2):350-352
16) Navrazhina K, Frew JW, Garcet S, Krueger JG. Epithelialized Tunnels Contribute to Inflammation in Hidradenitis Suppurativa. J Allergy Clin Immunol 2020; doi:10.1016/j.jaci.2020.12.651
17) Grand D, Frew JW, Navrazhina K, Krueger JG Doppler-Ultrasound Based Non-Invasive Biomarkers in Hidradenitis Suppurativa: Evaluation of Analytical and Clinical Validity. Br J Dermatol 2020; doi:10.1111/bjd.19343
18) Williams S, Frew JW, Krueger JG A Systematic Review and Critical Appraisal of Metagenomic and Culture Studies in Hidradenitis Suppurativa. Exp Dermatol 2020; doi:10.1111/exd.14141
19) Frew JW, Magnani L, Suarez-Farinas M, Garcet S, Brunner P, Czarnowicki T, Kim J, Bottomley C, Finney R, Cueto I, Fuentes-Duculan J, Omatsu H, Lentini T, Yanofsky V, Krueger JG, Guttman E. The Erythema Q Score: An Imaging Biomarker for Redness is Skin Inflammation Exp Dermatol 2020; doi:10.1111/exd.14224

20) Frew JW, Jiang CS, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger JG Dermal Tunnels Influence Time to Clinical Response and Family History Influences Time to Loss of Clinical Response in Hidradenitis Suppurativa Patients Treated with Adalimumab. Clin Exp Dermatol 2020; 46(2):306-313
21) Frew JW, Marzano A, Wolk K, Join-Lambert O, ALavi A, Lowes MA, Piguet V A Systematic Review of Promising Therapeutic targets in Hidradenitis Suppurativa: A Critical Evaluation of Mechanistic and Clinical Relevance. J Invest Dermatol 2021; 141(2):316-324
22) Frew JW. Hidradenitis Suppurativa is an Autoinflammatory Keratinization Disease: A Review of the Clinical, Histological and Molecular Evidence: JAAD International 2020;1(1):62-72
23) Krueger JG, Garcet S, Hawkes JE, Frew JW, Fuenetes-Duculan, Kunjravia N, Cueto I, Li X, Alonso-Llamazares J, Luo-Somolinos A, DuBois J, Singer J, Wachter E Immune Modulation in Psoriasis Lesions by Topical PH-10 (Rose Bengal Disodium) F1000 Research 2020;
24) Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, Ungar J, Krueger JG The Effect of Subcutaneous Brodalumab upon Clinical Disease Activity in Hidradenitis Suppurativa: An Open Label Cohort Study. J Am Acad Dermatol 2020; 83(5):1341-1348
25) Kamil MAA, Chandran NS, Sulistyaningrum SK, Rajagopalan M, Tahiliani S, Hsaio J, Naik HB, Shi V Frew JW The APHiS Foundation: Promoting Hidradenitis Suppurativa Research in the Asia Pacific. Int J Dermatol 2020; 59(8):289-290
26) Frew JW, Jiang C, Singh N, Grand D, Navrazhina K, Vaughan R, Krueger J “Malignancy and Infection Risk During Adalimumab Therapy in Hidradenitis Suppurativa” Clin Exp Dermatol 2020; 45(7):859-865
27) Price K, Frew JW, Hsiao J, Shi V “COVID-19 and Immunomodulator/Immunosuppressant Use in Dermatology” J Am Acad Dermatol 2020;82(5):173-175
28) Frew JW “Ant- Saccharomyces Cerevisiae Antibodies in Hidradenitis Suppurativa: More Than Just a Gut Feeling” J Aller Clin Immunol 2020; 146(2):458

29) Frew JW “Primary Imputation Methods Impact Efficacy Results in Hidradenitis Suppurativa Clinical Trials” J Am Acad Dermatol 2020; 83(2):663-665
30) Frew JW, Grand D, Navrazhina K, Krueger JG “Beyond Antibodies: B-cells in Hidradenitis Suppurativa: Bystanders, Contributors or Therapeutic Targets?" Exp Dermatol 2020; 29(5):509-515
31) Van Straalen KR, Frew JW “The Importance of Methodological Rigor in Proof-of-Concept Clinical Trials: A Lesson from Hidradenitis Suppurativa” J Invest Dermatol 2020; 141(2):429-431
32) Frew JW, Piguet V “Ex-Vivo Models and Interpretation of Mechanistic Studies in Hidradenitis Suppurativa” J Invest Dermatol 2020; 140(7):1323-1326
33) Grand D, Navrazhina K, Frew JW A Scoping Review of Non-Invasive Imaging Modalities in Dermatological Disease: Potential Novel Biomarkers in Hidradenitis Suppurativa. Front Med 2019; doi: 10.3389/fmed.2019.00253
34) Frew JW, Jiang C, Singh N, Grand D, Navrazhina K Vaughan R, Krueger JG Clinical Response Rates, Placebo Response Rates and Significantly Associated Covariates Are Dependent Upon Choice of Outcome Measure in Hidradenitis Suppurativa: A Post-Hoc Analysis of PIONEER 1 and 2 Individual Patient Data J Am Acad Dermatol 2019; 82(5):1150-1157
35) Grand D, Navrazhina K, Frew JW Integrating Complement into the Molecular Pathogenesis of Hidradenitis Suppurativa Exp Dermatol 2020; 29(1): 86-92
36) Frew JW Navrazhina K No Evidence that Impaired Notch Signaling Differentiates Hidradenitis Suppurativa from other Inflammatory Skin Diseases Br J Dermatol 2020;182(4):1042-1043
37) Phan K, Tatian A, Woods JA, Cains GD Frew JW Prevalence of Inflammatory Bowel Disease (IBD) in Hidradenitis Suppurativa (HS): Systematic Review and Adjusted Meta-Analysis Int J Dermatol 2019; DOI: 10.1111/ijd.14697
38) Navrazhina K Frew JW Krueger JG IL-17C is elevated in lesional Tissue of Hidradenitis Suppurativa Br J Dermatol 2020;182(4):1045-1047
39) Frew JW, Navrazhina K, In-Silico Analysis of Gamma-Secretase-Complex Mutations in Hidradenitis Suppurativa Demonstrates Disease-Specific Substrate

Recognition and Cleavage Alterations Front Med 2019; DOI: 10.3389/fmed.2019.00206

40) Byrd AS, Dina Y, Okoh UJ, Quartey QQ, Carmona-Rivera C, Williams DW, Kerna ML, Miller RJ, Petukhova L, Naik HB, Barnes LA, Shipman WD, Caffrey JA, Sacks JM, Milner SM, Aliu O, Broderick KP, Kim D, >iu H, Dillen CA, Ahn R, Frew JW, Kaplan MJ, Kang S, Garza LA, Miller LS, Alavai A, Lowes MA, Okoye GA Specimen Collection for Translational Studies in Hidradenitis Suppurativa Sci Rep 2019;9(1):12207

41) Frew JW, Murrell DF Improving Clinical Applications of Quality of Life Scores in Epidermolysis Bullosa (EB): Defining Clinically Significant Outcomes in the QOLEB Questionnaire Mucosa 2019; doi: 10.33204/mucosa.598339

42) Frew JW, Navrazhina K, Byrd AS, Garg A, Ingram JR et al Defining Lesional, Perilesional and Unaffected Skin in Hidradenitis Suppurativa: Proposed Recommendations for Clinical Trials and Translational Research Studies Br J Dermatol 2019; 181(6):1339-1341

43) Frew JW, Navrazhina K, Maroun M, Lu PJ, Krueger JG Contribution of Fibroblasts to Tunnel Formation and Inflammation in Hidradenitis Suppurativa Exp Dermatol 2019 28(8):886-891

44) Tatian A, Slape D, Lawless R, Frew JW A Systematic Review of Reported Cases of Pachyonychia Congenita Tarda Clin Exp Dermatol 2019; 44(6):606-612

45) Frew JW The contradictory Inefficacy of Methotrexate in Hidradenitis Suppurativa: A Need to Revise Pathogenesis or Acknowledge Disease Heterogeneity? Journal Dermatol Treatment 2019; 31:422-423

46) Frew, J. W. (2019), Hidradenitis suppurativa and diabetes: big data bias masks a true association. Clin Exp Dermatol. doi:10.1111/ced.13954

47) Frew JW (2019) The Hygiene Hypothesis, Old Friends, and New Genes. Front. Immunol. 10:388. doi: 10.3389/fimmu.2019.00388

48) Frew JW “Commentary: Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model” Frontiers in Immunology 2019; 2019; 10: 302

49) Frew JW, Hawkes JE, Sullivan-Whalen M, Gilleaudeau P, Krueger JG “Inter-Relater Reliability of Phenotypes, and Exploratory Genotype- Phenotype Analysis in Inherited Hidradenitis Suppurativa” Br J Dermatol 2019; 2019 Jan 28. doi: 10.1111/bjd.1769
50) Frew JW “Assessing the Efficacy of New Biologic Therapies in Hidradenitis Suppurativa: Consistency vs Bias in Moderate and Severe Disease” JEADV 2019 Mar 15. doi: 10.1111/jdv.15572
51) Frew JW, Hawkes JE, Krueger JG “A Systematic Review and Critical Evaluation of Inflammatory Cytokine Associations in Hidradenitis Suppurativa” F1000Research (2018); 2018 Dec 13;7:1930
52) Frew JW, Hakes JE, Krueger JG “A Systematic Review and Critical Evaluation of Immunohistochemical Associations in Hidradenitis Suppurativa” F1000Research (2018); 2018, 7:1923
53) Frew JW, Hawkes JE, Krueger JG “Topical, Systemic and Biologic Therapies in Hidradenitis Suppurativa: Pathogenic Insights Through Examination of Therapeutic Mechanisms” (2018) Therapeutic Advances in Chronic Disease 2019; 10: 2040622319830646.
54) Shavit E, Alavi A, Bechara FG, Bennett RG, Bourcier M, Cibotti R, Daveluy S, Frew JW et al “Proceeding Report of the Second Symposium on Hidradenitis Suppurativa Advances (SHSA) 2017”.(2018) Exp Dermatol 2019 Jan;28(1):94-103
55) Frew JW, “Complement, Hidradenitis Suppurativa and Pathogen-Induced Positive Selection” Br J Dermatol 2018; doi:10.1111/phpp.12436
56) Slape D, Tang J, Lawless R, McCrossin I, Frew JW “A Retrospective Cohort Study of Melanoma Prevalence Stratified by Body Site in a Regional Australian Population 1994-2017: Site-Specific Protective Mechanisms” (2018) Photodermatol Photoimmunol Photomed 2018; 35(3):135-140
57) Frew JW “We Need to Talk About Notch: Notch Dysregulation as an Epiphenomenon in Inflammatory Skin Disease” (2018) Br J Dermatol 2019 Feb;180(2):431-432

58) Siva T, Choy B, Satchell AC, Woods JA, Frew JW (2018) “Secukinumab in Pityriasis Rubra Pilaris: A Case Series Demonstrating Variable Clinical Response and the Need for Minimum Clinical Datasets” (2018) JAAD Case Reports 2018;4: 500-505
59) Frew JW, Davidson M, Murrell DF (2017) “Disease-Specific Health Related Quality of Life Patient Reported Outome Measures in Genodermatoses: A Systematic Review and Critical Evaluation” Orphanet Journal of Rare Diseases 2017; 12:189
60) Vekic DA, Frew JW (2017) “PBS Listing of Adalimumab for Hidradenitis Suppurativa: A Life-Changing Drug or does Lifestyle Change the Drug?” Australas J Dermatol 2018;59:e243-e244
61) Frew JW (2017) “Quality of Life Measures in Hidradenitis Suppurativa: The importance of high quality methodology and standards” J Cutan Med Surg 2018; 22:368
62) Vekic DA, Frew JW, Cains GD (2017) “Hidradenitis Suppurativa, a review of pathogenesis, associations and management. Part 1” Australas J Dermatol 2017; 10.1111/ajd.12770
63) Kim E, McCrossin I, Frew JW (2017)“HLA-B*1502 haplotype screening prior to carbamazepine administration in individuals of southeast Asian ancestry nears cost effectiveness in preventing severe cutaneous adverse drug reactions” Aust J Dermatol 2018;59:245-246
64) Frew JW, Vekic DA, Woods J, Cains GD (2017) “Drug Associated Hidradenitis Suppurativa: A Systematic Review of Case Reports” J Am Acad Dermatol 2018;78(1):217-219
65) Frew JW, Vekic DA, Woods J, Cains GD (2017) “A Systematic Review and Critical Evaluation of Reported Pathogenic Sequence Variants in Hidradenitis Suppurativa” British Journal of Dermatology 2017; DOI:10.1111/bjd.15441
66) Rademaker M, Agnew K,Andrews M, Armour K, Baker C, Foley P, Frew JW, Gebauer K, Gupta M, Kennedy D, Marshman G, Sullivan J (2017)“Psoriasis in those planning a family pregnant or breast-feeding. The Australasian Psoriasis Collaboration” Australasian Journal of Dermatology DOI:10.1111/ajd.12641

67) Frew JW Murrell DF (2017)“Highly Resistant Acrodermatitis Continua of Hallopeau and Pustular Psoriasis” Skin Appendage Disorders 2017; DOI:10.1159/000464140
68) Frew JW, Fallah H (2017)“Lichen Amyloidosis Involving the Scalp” Australasian Journal of Dermatology 2017 DOI:10.1111/ajd.12582
69) Frew JW (2017)“Beyond the tip of the Iceberg: the role of phosphoinositide 3-kinase/AKT in acne inversa/hidradenitis suppurativa” British Journal of Dermatology 2016:174(3): 484-485
70) Frew JW, Henderson JA, McCrossin I (2016) “The Early Bird and The Worm: A Case of Cercarial Dermatitis” The Medical Journal of Australia 2016; 204(3): 122:123
71) Vekic DA, Frew JW, Woods J, Cains GD (2016) “Adopting the Orphan”: The Importance of Recognising Hidradenitis Suppurativa as a Systemic Auto-inflammatory Disease” Australas J Dermatol. 2016;57(1):69-70
72) Zhao CY Frew JW Muhaidat J Cheung K Lee P Poulos V McCrossin I Cachia AR Tefany F Murrell DF (2016) “Chlorophyll-induced pseudoporphyria with ongoing photosensitivity after cessation – a case series of four patients” Journal of the European Academy of Dermatology and Venereology 2016;30(7):1239-42
73) Frew JW, Nguyen RTD (2015) “Tattoo-Associated Mycobacterial Infections: An Emerging Public Health Issue” The Medical Journal of Australia 2015; 203(5):223
74) Frew JW, Vekic DA, Woods J Cains GD (2015) “Phenotypic Heterogeneity Implies Heterogeneous Pathways in Hidradenitis Suppurativa” Exp Dermatol 2015; 24(5): 338-9
75) Frew JW, Murrell DF, Haber RM (2015) “50 Shades of Yellow: A Review of Xanthodermatology” Int J Dermatol. 2015;54(10):1109-23
76) Frew JW, Parsi K (2015) “Adalimumab Induced Porokeratosis” Aust J Dermatol 2015; 56(3):E80-1
77) JW Frew (2015) “Multinucleate Cell Angiohistiocytoma: Clinco-Pathological Correlation of 142 Cases with Insights into Aetiology and Pathogenesis” Am J Dermatopathol 2015; 37(3):222-8

78) Frew JW (2014) “Exploring the Evidence: Clinically Relevant Drug Interactions between Psychoactive and Dermatological Medications”. Treatment Strategies Dermatology 2014 4(1):37-42

79) Yuen WY, Frew JW, Veerman K Van Den Heuvel ER Murrell DF, Jonkman MF (2014) “Health Related Quality of Life in Epidermolysis Bullosa: Validation of the Dutch QOLEB Questionnaire and Assessment in the Dutch Population” Acta Derm Venerol 94(4): 442-447

80) Venugopal SS, Yan W, Frew JW, Cohn HI, Rhodes LM, Tran K, Melbourne W, Nelson JA, Sturm M, Fogarty J, Marinkovich MP, Igawa S, Ishida-Yamamoto A, Murrell DF (2013) “A Phase II randomised vehicle-controlled trial of intradermal allogeneic fibroblasts for recessive dystrophic epidermolysis bullosa” J Am Acad Dermatol 69(6): 898-908

81) Tjokrowidjaja A[1], Daniel BS, Frew JW, Sebaratnam DF, Hanna AM, Chee S, Dermawan A, Wang CQ, Lim C, Venugopal SS, Rhodes LM, Welsh B, Nijsten T, Murrell DF. (2013) “The Development and Validation of the Autoimmune Bullous Disease Quality of Life Questionnaire: A tool to measure the quality of life impacts of treatments used in patients with autoimmune blistering disease” Br J Dermatol 169(5):1000-6

82) Sebaratnam DF[1], Hanna AM, Chee SN, Frew JW, Venugopal SS, Daniel BS, Martin LK, Rhodes LM, Tan JC, Wang CQ, Welsh B, Nijsten T, Murrell DF. (2013) “Development of a Quality of Life Instrument for Autoimmune Bullous Disease: The Autoimmune Bullous Disease Quality of Life Questionnaire” JAMA Dermatology 149(10):1186-91

83) Frew JW, Cepeda Valdez R, Fortuna G, Murrell DF, Salas Alanis JC (2013) “Measuring Quality of Life in Epidermolysis Bullosa in Mexico: Cross Cultural validation of the Hispanic Version of the Quality of Life in Epidermolysis Bullosa questionnaire”. Journal of the American Academy of Dermatology 69(4): 652-653

84) Sebaratnam DF, Venugopal SS, Frew JW, McMillan JR, Finkelstein ER, Martin LK, Murrell DF. (2013) “Diffuse Melanosis Cutis: A Systematic Review of the Literature” Journal of the American Academy of Dermatology 2013; 68(3): 482-8

85) JW Frew (2013) “The Clinical Significance of Drug Interactions between Dermatological and Psychoactive Medications” Dermatologic Therapy 2014;27(1):1-11

86) KJ Purdie, C Pourreyron, H Fassihi, R Cepeda-Valdes, JW Frew, A Volz, SJ Weissenborn, H Pfister, CM Proby, L Bruckner-Tuderman, DF Murrell, JC Salas-Alanis, JA McGrath, IM Leigh, CA Harwood and AP South. (2010) “No Evidence that Human Papillomavirus is Responsible for the Aggressive Nature of Recessive Dystrophic Epidermolysis Bullosa-Associated Squamous Cell Carcinoma” Journal of Investigative Dermatology 2010; 130(12): 2853–2855.

87) J.W. Frew, S-W Lim, A Klausseger, CW Chow, K Tran, J. Su, D. Orchard, H. Shimizu, D. F. Murrell (2010) “Autosomal Dominant Bullous Dermolysis of the Newborn Associated with a Heterozygous Misense Mutation p.G1673R in Type VII Collagen” Australasian Journal of Dermatology 2011;52(4):e1-4

88) J.W. Frew, P.J.H. Dopping-Hepenstal, J.A McGrath (2010) “Categorizing Immunoflourescence mapping in Epidermolysis Bullosa with Pyloric Atresia: Use as a broad prognostic indicator.” Australasian Journal of Dermatology. 2011;51(3):212-4

89) J.W.Frew, L.K. Martin T. Nijsten, D.F. Murrell (2009) “Quality of Life Evaluation in Epidermolysis Bullosa (EB) through the development of the QOLEB Questionnaire: an EB-Specific Quality of Life Instrument” British Journal of Dermatology (2009) Dec 161(6):1323-30

90) J.W. Frew (2009) “Performing Surgery with a Single Electron: Electrosurgery and Quantum Mechanics" Australian and New Zealand Journal of Surgery (2009) Oct 79(10):680-2

91) MA Nading; JJ Lahmar; JW Frew; N Ghionis; M Hanley;A Kemble Welch, DF Murrell,(2009) “A ski and adventure camp for young patients with severe forms of Epidermolysis Bullosa” Journal of the American Academy of Dermatology 60(3):AB5

Book chapters and other Non-Peer-Reviewed Papers

1. Frew JW “Biomarkers in Hidradenitis Suppurativa: One Step Closer to the Clinic” Br J Dermatol 2021;185(4):696-697
2. Frew JW “Follicular Occlusion and Inflammation:” in A Comprehensive Guide to Hidradenitis Suppurativa 1e- Elsevier 2021
3. Frew JW, Grand D, Navrazhina K, Hawkes JE “Research Pipeline I: Oral Therapeutics for Psoriasis” in Advances in Psoriasis; Springer 2020.
4. Grand D, Frew JW, Hawkes JE “Epigenetics of Atopic Dermatitis: Mechanisms Beyond The Exome” in Atopic Dermatitis: Inside Out or Outside In? Cambridge Scholars Publishers 2020;
5. JW Frew “Putting Nodule Counts Behind Us: Hidradenitis Suppurativa Outcome Measures Independent of Descriptive Semantics” Br J Dermatol 2019; 182(4)
6. K Navrazhina, JW Frew “Consensus Definitions of Disease Activity in HS: Crossing the Semantic Gap” Br J Dermatol 2019; 182(1):7-7
7. Frew JW “Lack of photographic documentation undermines assessment of hidradenitis suppurativa phenotypes: reply from the author” Br J Dermatol 2019; 181(1):225
8. D Grand, K Navrazhina, JW Frew JE Hawkes “Research Pipeline II: Oral Therapeutics for Psoriasis” Elsevier
9. JWJ Lasschuit, Snaith JR, Frew JW “The Skin and Diabetes” in “Skin and the Heart” 1st Edition Edited by DF Murrell and J Otton Springer Press
10. JW Frew K Navrazhina “Into the (Gluteal) Fold: Pilonidal Disease and Hidradenitis Suppurativa – Association or Continuum?” Br J Dermatol 2019; 181(6):1121
11. JW Frew (2019) “Hidradenitis Suppurativa and Other Related Disorders” Stiehm’s Immunodeficiencies, 2nd Edition edited by Kathleen Sullivan and Richard Steihm.
12. JW Frew (2018) “New Therapeutics for Itch in Dermatomyositis” Br J Dermatol 2018;179(3):559-560
13. JW Frew (2018) “Children of the (Translational) Revolution” Br J Dermatol 2018; (Accepted for Publication 29th March 2018)

14. JW Frew, A Anstey (2017) “BJD Research Letters: Concise, Thought Provoking and of General Interest” Br J Dermatol 2018; (Accepted for Publication 9th Nov 2017)
15. JW Frew, DF Murrell (2013) “Autoimmune Blistering Diseases and Corticosteroid Use: A Review of the Evidence” Blistering Diseases: Clinical Features, Pathogenesis, Treatment, 1st Edition edited by Dedee F Murrell, 01/2015: chapter Autoimmune Blistering Diseases and Corticosteroid Use: A Review of the Evidence: pages 459-468; Springer.
16. JW Frew, DF Murrell (2013) “Living with Epidermolysis Bullosa: A Review of the Impact on Individuals’ Quality of Life” Blistering Diseases: Clinical Features, Pathogenesis, Treatment, 1st Edition edited by Dedee F Murrell, 01/2015: chapter Autoimmune Blistering Diseases and Corticosteroid Use: A Review of the Evidence: Springer.
17. JW Frew, C Chams-Davatchi, DF Murrell (2013) “Cyclophosphamide in Autoimmune Blistering Diseases: Safety, Efficacy and Evidence Base” Blistering Diseases: Clinical Features, Pathogenesis, Treatment, 1st Edition edited by Dedee F Murrell, 01/2015: pages 507-513; Springer
18. JW Frew, DF Murrell (2012) “Corticosteroid Use in Autoimmune Blistering Diseases” Immunol Allergy Clin North Am. 32(2):283-294
19. JW Frew, LK Martin, DF Murrell (2011) “Evidence Based Medicine in Treatment of Pemphigus Vulgaris and Pemphigus Foliaceus” Dermatology Clinics of North America 29(4): 599-606
20. JW Frew, DF Murrell (2011) “Current Management Strategies in Paraneoplastic Pemphigus (Paraneoplastic Autoimmune Multiorgan Syndrome- PAMS)” Dermatology Clinics of North America 29(4): 607-612
21. JW Frew, DF Murrell (2011) “The Use of Corticosteroids in Autoimmune Blistering Diseases” Dermatology Clinics of North America 29(4): 535-544
22. JW Frew, DF Murrell (2011) “Paraneoplastic Pemphigus (Paraneoplastic Autoimmune Multiorgan Syndrome): Clinical Presentations and Pathogenesis” Dermatology Clinics of North America 29(3): 419-425

23. J.W. Frew and D.F. Murrell (2010) “Quality of Life Measurements in Epidermolysis Bullosa: Tools for Clinical Research and Patient Care” Dermatology Clinics (2010) 28: 185-90
24. J.W. Frew, D.F. Murrell (2007) “Acanthosis Nigricans” Medical Observer 20 April 2007.
25. J.W. Frew, K. Tran, D.F. Murrell (2006) “Patches Herald Pityriasis Rosea” Medical Observer 24 Nov 2006.