Advancements and Areas of Need in Paediatric Liver disease, Intestinal and Pancreatic Transplantation

By

Michael R Couper

MBChB, FRACP

Thesis

submitted to Flinders University

for the degree of

PhD by Published Works

College of Medicine and Public Health

Approved 30th April 2025

TABLE OF CONTENTS

THESIS SUMMARY

The pathology of paediatric liver disease is highly heterogeneous with frequent innovative advances and incremental improvements in diagnostics and care. Transplantation, where not contraindicated, remains the primary treatment for irreversible acute liver failure and chronic liver disease regardless of underlying aetiology. Transplantation is also utilised similarly for irreversible intestinal failure particularly with TPN complications and for chronic debilitating pancreatitis, for a reduction in associated symptom morbidity, to preserve islet cell function and prevent pancreatic adenocarcinoma.

This thesis presents nine publications, seven within the body and 2 within appendices to the thesis, to explore the themes of advancements in liver and intestinal and pancreatic transplantation and areas of ongoing need. A Contextual Statement discusses background, explores the extant literature, and story-boards the thesis papers, highlighting original contributions to knowledge and clinical practice. Journal metrics and impacts further explore the importance of these works to the field. There then follows an exploration of themes in three subsections as follows:

Chapter 2, Section 1 – Novel Aetiologies

Three publications explore changes in new emerging and poorly described diagnostic entities within the field. The first paper explores a new high-morbidity form of acute liver disease that has emerged in children post-COVID and the second a form of genetic liver disease with previously unexplored pathogenesis and unique management needs. The third paper details the diagnosis and management of a rare form of tumour which can involve the liver, gastrointestinal tract, and pancreas, proceeding to discuss the future of malignancy screening programs.

Chapter 2, Section 2 – Areas of Need and Further Research Within the Specialty

Two publications supplemented with one appendix explore areas of need within Paediatric Hepatology. Drug-induced liver disease (DILI) is discussed in the context of a description of a severe reaction resulting in transplantation in a child. Biliary disease, an area where therapeutic options are limited, is discussed in the context of a rare biliary lesion mimicking more common pathology. An appendix is included which examines the changing picture of neonatal liver disease over the last three decades with recommendations as to approach changes in both diagnosis and prognosis.

Chapter 2, Section 3 – New and Emerging Management Strategies

Two publications supplemented with one appendix explore new techniques for managing patients with liver, intestinal and pancreatic auto-islet transplantation. The first paper details the use of bedside ultrasound for monitoring rejection in children post-intestinal transplantation, a small group with complex needs and high morbidity. The second paper explores outcomes and considerations in children requiring more than one hepatic transplant, a growing patient group with the adoption of modern transplant techniques and immunosuppression regimes. An appendix details the use of islet cell transplantation (TP-IAT) for managing hereditary pancreatitis—an area of need, particularly in South Australia, the authors' practice location with demonstrated high prevalence.

In summary, the 7 publications presented in this thesis supported by 2 appendices, both directly and as a framework, explore the themes of advancement and areas of need in this area. They discuss changes in diagnostic trends, new post-COVID and genetic diagnostic entities, challenging areas (DILI, Biliary disease, and Neonatal Liver Failure), and promising innovations in transplant patient management (TP-IAT, retransplantation and point-of-care ultrasound).

CAREER REVIEW

I graduated from Otago University medical school in 2010. Following internship and a senior house surgeon year in New Zealand, I initially underwent training in Radiology in Australia successfully completing the training program's part one examinations in 2013. I opted to swap to Paediatric training the year following passing the colleges examinations in 2016 and commencing Gastroenterology training in 2017.

Throughout my training I have had an interest in hepatology and transplantation having undergone fellowships at all the Australian liver / intestinal transplantation centres (Royal Children's Hospital, Melbourne, Children's Hospital at Westmead, Sydney and Brisbane Children's Hospital, Brisbane). Additionally, I underwent a fellowship in 2022 at Birmingham Children's Hospital, UK with the hospital's liver unit. During this period the United Kingdom experienced a hepatitis of unknown cause epidemic and eventual pandemic resulting in a surge of hepatitis presentations and liver transplantations. Research during and after these attachments has remained a strong priority (See Appendix – Author Bibliography pg 112).

Inspired by Radiology training, point of care ultrasound has remained an area of interest for me. In 2021 I received training in and achieved qualification in bedside ultrasound with the Gastroenterology network of Intestinal Ultrasound (GENIUS) as well as the International Bowel Ultrasound society (IBUS). This training focused on bed side ultrasound assessment of Inflammatory bowel disease, however, I have applied it to both the management of constipation as well as in research for monitoring in children post intestinal transplantation.

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my many mentors over the years as well as my co-authors. Without your support and help I would never have reached this milestone.

A particular special mention of Dr Chayarani Kelgeri, a mentor, supervisor, and coauthor on nearly half the papers included in this thesis. Thank you for your endless support and selflessly helping me to achieve so much in such a short time despite conflicting demands for time.

Finally, I would like to thank my family and loved ones. You keep me anchored and no achievements would be worth it without you there to share them with.

Nothing in this world that's worth having comes easy

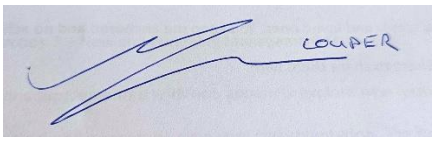
– Dr Bob Kelso, Scrubs, Season 4, Episode 20

DECLARATION

"I, Michael Couper, certify that this thesis:

1. Does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university
2. And the research within will not be submitted for any other future degree or diploma without the permission of Flinders University;
3. 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."
4. I confirm that I am a recipient of RTP support and acknowledged the contribution of an Australian Government Research Training Program Scholarship in this thesis

Signed –



Date – 13.2.25

ABBREVIATIONS

AAV – Adeno-associated virus

ALF – Acute liver failure

ALT – Alanine aminotransferase

CMV - Cytomegalovirus

CT – Computed Tomography

DILI – Drug induced liver injury

FISH – Fluorescence in situ hybridization

GALD – Gestational alloimmune liver disease

GLIS3 – GLI-similar zinc finger 3

HHV6 – Human Herpes virus 6

HLH – Hemophagocytic Lymphohistiocytosis

HSV – Herpes Simplex virus

IMT – Inflammatory Myofibroblastic tumours

IEQ/Kg – Islet equivalent number per kilogram body weight

IvIG – Intravenous Immunoglobulin

MPS - Mucopolysaccharidosis

MRI – Magnetic resonance imaging

NICU – Neonatal intensive care unit

NSAIDS – Non steroidal anti-inflammatory drugs

NTBC - Nitisinone

NLF – Neonatal Liver Failure

OMIM – Online Mendelian Inheritance in Man

PICU – Paediatric Intensive care unit

PSC – Primary Sclerosing Cholangitis

PT – Prothrombin time

SARS-CoV2 – Coronavirus disease 19

SA – South Australia

TCDD - 2,3,7,8-Tetrachlorodibenzo-p-dioxin

TPN – Total Parenteral nutrition

TP-IAT – Total Pancreatectomy with Islet Auto transplantation

CHAPTER 1 – CONTEXTUAL STATEMENT

1. INTRODUCTION AND BACKGROUND

Paediatric hepatology first emerged as a distinct entity in the 1960s. Most of the focus at the time was on diagnosis and therapeutic options were limited. There have been quantum leaps in both diagnostic and therapeutic armamentarium. These include liver transplantation, an appreciation of viral hepatides with new and emerging diseases, and the use of molecular and genetic techniques to expand our diagnostic capabilities, design therapeutic strategies and understand normal physiology, and improve monitoring techniques. Some of the later have been applied to small intestinal transplantation. A similar expansion has been seen in the management of Paediatric pancreatic disease beyond traditional exclusively supportive cares. I will discuss these in this thesis.

The liver has a limited capacity to regenerate and the end result of liver damage often will be liver transplantation. The major aetiologies for liver transplant in children are obstructive liver disease, especially biliary atresia, acute liver failure due to viruses, toxins and drugs, liver disease of unknown aetiology, autoimmune and metabolic liver diseases (1). The advent of rapid genetic sequencing techniques as well as the development of transplantation and referral databases has allowed the identification of new conditions and the development of new management stratagems and algorithmic paradigms. Additionally, the SARS-CoV2 epidemic of 2020 changed the health landscape. While acute cases typically involved presentation with severe acute respiratory syndrome, a subsequent severe hepatitis epidemic in 2022 in children under 6 years is suspected to be related and represents a new diagnostic entity (2).

Transplantation outcomes and individualised protocols for specific conditions have developed over time, however there are still areas of substantial need (3). Paracetamol overdose is the most common drug induced liver injury (DILI) and robust guidelines and clinical outcome calculators have been created to guide management. Most patients can be treated with N-Acetylcysteine and

transplantation is rarely required (4). DILI due to other agents is common, but in contrast to paracetamol there have been few studies in children, with resulting challenges for prognostication and transplantation planning (5). Biliary atresia is the most common indication for liver transplantation in children. Clear guidelines exist regarding diagnosis as well as optimal timing for Kasai hepatic portoenterostomy. Meanwhile the management of primary sclerosing cholangitis and other autoimmune biliary diseases remains challenging and is predominantly limited to supportive care. The pathogenesis of these conditions remains poorly understood.

Outcomes following liver transplantation have dramatically improved mostly due to contemporary immunosuppression regimes. While both patient and graft survival in liver transplantation has improved over time, 18.4% of patients will develop graft failure within the first 10 years post-transplant (6). Studies have demonstrated similar outcomes in children receiving a second transplant however, with the younger age of patients, 15% of retransplants will require further subsequent grafts (6). There is a lack of data on long term outcomes to help guide management in these patients. Both the unique management challenges as well as the ethical consideration of retransplantation remain largely unexplored and problematic.

Intestinal transplantation is indicated in children with intestinal failure dependent on total parental nutrition who fail intestinal rehabilitation or develop life threatening complications such as liver disease, recurrent line sepsis or loss of vascular access (7). Fortunately, the use of intestinal rehabilitation and GLP-2 agonists have reduced the number of children who meet these criteria. These children represent a small subset of patients with unique and complex medical needs. Intestinal grafts are highly immunogenic due to large amounts of lymphoid tissue, abundant epithelial cells and constant exposure to external antigens and microbiota (8). Acute rejection is common affecting 50 – 75% of patients and monitoring requires frequent anaesthetics to obtain graft biopsies (9). New management and monitoring strategies are needed to reduce morbidity and increase both graft and patient survival.

This thesis aims to explore advancements and areas of need in paediatric liver disease, intestinal and pancreatic transplantation through three sections, Novel Aetiologies, Areas of Further Need within the Specialty, and, New and Emerging Management Strategies. An introduction follows providing an overview on the area as well as the field of knowledge within current literature.

1.1. Traditional aetiologic groups of Paediatric liver disease

The liver is a complex organ with numerous physiological functions including bile production and secretion, metal, vitamin, and glycogen storage, protein synthesis and nitrogenous waste disposal. This results in an expansive range of aetiologies that can result in childhood liver disease. This section aims to provide background on conditions and concepts discussed in this thesis.

Biliary atresia is the most common cause of cholestatic jaundice in infancy, outside of the preterm population and beyond the neonatal period, as well as the most common indication for liver transplantation in childhood. It is a progressive obliterative cholangiopathy affecting the extrahepatic bile ducts presenting during infancy. Incidence ranges from 1 in 5000 to 1 in 19,000 births varying with geographic location (10). Increased risk has been reported with infants conceived in spring, low intakes of vitamin E, copper, phosphorus, and beta tocopherol (11). Increased incidence is seen in French Polynesian, subcontinental, and Inuit populations (12). While pathogenesis remains unclear this suggests an interplay of genetic and environmental factors. Diagnosis is established based on a suggestive clinical picture in an infant with cholestatic jaundice and acholic stools. Ultrasonographic and biliary scintigraphy appearances can assist with confirmation by biopsy and intraoperative cholangiogram. Kasai hepatic portoenterostomy is effective in reestablishing bile flow if performed within the first 60 days of life (70% effective) however success is greatly diminished beyond 90 days of life (<25% effective) (13). Most patients will require liver transplantation with only 22% reaching adulthood with their native liver (14).

The management of biliary atresia is distinctly different to other common biliary lesions. Primary sclerosing cholangitis for example similarly presents with a biliary obstructive picture, with an increased risk in patients with a family history, inflammatory bowel disease and autoimmune conditions (15). Management is supportive only unless transplantation becomes indicated. The spectrum between established sclerosing cholangitis and other autoimmune conditions of the small bile ducts is not well understood and further research into aetiology and management strategies are needed.

Infectious aetiologies are the most common cause of acute hepatitis in children globally primarily due to the hepatotropic viruses, Hepatitis A-E. Hepatitis A and E only cause acute liver injury with supportive care sufficing following serological diagnosis, and complete recovery being the rule. Fulminant hepatitis with acute liver failure is rare but can be fatal. Both viruses are shed in the stool of infectious individuals with transmission usually through contaminated food or drinking water. In the context of a predominantly self-limiting illness, the best management is preventative with vaccination. Since the introduction of routine childhood vaccination, the incidence of Hepatitis A in the United States between 1995 to 2007 dropped 92% in children under 15 years of age. Hepatitis E represents a leading cause of acute hepatitis particularly in developing nations with increased risk in lower socioeconomic status, higher population densities and with shellfish intake ((16),(17)). A vaccine is available and while recommended by the World Health Organisation for outbreak containment it is only currently licensed for use in China (18).

Hepatitis B and C are blood borne viruses that can cause chronic infection with subsequent chronic cirrhotic liver disease and hepatocellular carcinoma being the main concerns. These serious complications usually take decades to develop and are rare in children. The main source of infection in childhood is perinatal transmission. In the case of Hepatitis B this can be prevented with Hepatitis B immunoglobulin prophylaxis and vaccination which is effective in 97% (19). All children with chronic Hepatitis B need annual monitoring and those with persistent infection or disease flares

should be considered for anti-viral therapy. Antiviral treatment is not curative but aims to suppress viral replication and prevent progress to cirrhosis and hepatocellular carcinoma (20). Hepatitis C is an RNA virus with a high degree of heterogeneity and a high rate of mutation. Its genetic diversity allows it to avoid immune surveillance and makes the development of an effective vaccine challenging (19). Direct-acting antiviral treatment is curative in > 95% of individuals (21). Liver transplantation when indicated is performed for both Hepatitis B and C however is very rare in childhood. Recurrence in the graft is almost universal in Hepatitis C and in Hepatitis B unless prevented with antivirals and Hepatitis B immunoglobulin (19). Hepatitis D is a viral symbiont which can cause severe disease with high rates of progression to cirrhosis in association with Hepatitis B. This is most unusual in children (22).

A large array of other infective agents cause acute childhood hepatitis including Enterovirus, Cytomegalovirus, Ebstein-Barr Virus, Herpes Simplex Virus, Adenovirus, Human Herpes Virus 6/7, Parvovirus, Sapovirus and Norovirus, as well as the bacterial infections Leptospirosis and Brucellosis and various parasite infections such as Schistosomiasis (23). Treatment in most viral hepatitis cases is supportive. Adenovirus and Adeno-associated virus have both been implicated in the 2022 hepatitis epidemic in children under 6 years of age in the northern hemisphere. Prior to this event Adenovirus hepatitis had been almost always described in immunosuppressed children and neonates only and Adeno associated virus was not known to cause disease in humans ((24), (25)). Diagnostic trends, management strategies and aetiologies for this recent epidemic will be discussed further in this thesis.

The liver is a key metabolic organ with roles in energy production, peptide synthesis, metal storage and waste processing. These numerous complex pathways result in a wide spectrum of childhood genetic and metabolic diseases resulting in liver disease or necessitating transplantation. Classic conditions include disorders of amino acid and peptide metabolism (Tyrosinemia, Citrullinemia and OTC deficiency), carbohydrate metabolism (Galactosemia, hereditary fructose intolerance), peptide

synthesis (Alpha-1-Antitrypsin deficiency), lysosomal metabolism (Niemann-Pick disease type C, Lysosomal acid lipase deficiency), mitochondrial and disorders of fatty acid oxidation, and peroxisomal disorders (Zellweger's syndrome). Traditionally diagnosis was made based on a suggestive clinical presentation as well as metabolic screening tests predominantly ammonia, lactate, amino acids profile, acylcarnitine profile, triglycerides, cholesterol, transferrin isoforms, very long chain fatty acids and urinary organic acids.

Confirmation of metabolic liver disease is via genetic testing and increasingly in cases with a suggestive presentation this is superseding traditional screening tests. The first application of clinical genetic analysis was in the late 1950s with the recognition of trisomy 21 in patients with Down's Syndrome on karyotype. In the early 1990s and 2000s fluorescence in situ hybridization (FISH) was developed and in conjunction with the human genome project allowed the development of probes for specific diagnostic entities. Subsequently comparative genomic hybridisation was developed permitting the detection of chromosomal copy number changes without the need for cell culturing addressing the time consuming and expense of the whole genome approach in FISH while also increasing resolution. The downside of these techniques is their inability to detect balance aberrations and single nucleotide polymorphisms. With the establishment of the polymerase chain reaction technique, molecular genetic techniques were rapidly developed in the 1990s. With the advent of massively parallel sequencing (MPS) and sequence automatization the cost and speed of whole exome and genome sequencing has exponentially decreased. The original human genome sequencing project involving the National Institute of Health in collaboration with 20 other groups took 11 years to complete. Current next generation sequencing at the time of writing allows whole genome sequencing for as little as US$1000 within 3 – 5 days with future improvement projected to reduce the cost and processing time to a fraction of this allowing the possibility of routine screening (26).

Rapid whole exome sequencing is already proven to be an effective diagnostic tool in critically ill infants and the wide spread application of sequencing has allowed the development of catalogues and databases such as the Online Mendelian Inheritance in Man (OMIM). Widespread use or rapid genetic testing has resulted in the recognition of a wide range of rare and novel conditions and has become critical to rapidly establish diagnose in infants. This thesis will discuss rare conditions now recognised due to genetic techniques and how experience and understanding in these conditions has led to individualised management for conditions as well as an understanding of their pathogenesis.

Drug induced liver disease (DILI) is an underdiagnosed and under researched area of liver disease particularly in paediatrics. This is disproportionate to its impact. Rates are not known in children however in the adult population 57% of cases of acute liver failure are due to paracetamol overdose and a further 11% due to non-paracetamol drugs. DILI is classified as intrinsic, dose dependent and affecting all individuals, or idiosyncratic, dose independent and as a result of an interaction within susceptible individuals (5). Paracetamol overdose is the most common dose dependent DILI and is well studied with robust protocols for commencement of treatment with N-Acetylcysteine which if administered in a timely and appropriate fashion is nearly 100% effective. Additionally scoring systems exist to help guide prognosis and need for liver transplantation (4). Paracetamol induced DILI is on the increase with a 44.3% increase in cases 2016-17 as compared with 2007-08 in Australia (27). While clear data is not available non paracetamol DILI also appears to be on the rise with worse 90-day transplant free survival (28). Certain groups of medications are recognised as leading causes of idiosyncratic DILI in children, predominantly antimicrobials (51%) and antiepileptics (21%) (5). The challenge stems from the wide range of pathophysiology through which DILI can arise including mitochondrial impairment, cholestasis, lysosomal impairment, hepatocellular damage from reactive metabolites and dysregulation of the immune system. Management in most cases is nonspecific and involves removing the offending agent and supportive care. However, up to 17% can develop chronic liver or end-stage liver disease (5). If recovery occurs it can be quite delayed. Prognostic indicators to help guide management decisions are not available in children. We need to elucidate the

mechanisms which result in hepatocellular injury for various drugs and use this to develop personalised treatments. This thesis looks to explore this idea through a description of transplantation in a child following Lamotrigine overdose providing insight into the pathogenesis of idiosyncratic injury for this agent.

Liver and intraabdominal neoplasms make up a small but not insubstantial component of the overall cohort who require listing for liver transplantation (4% of paediatric transplantations in Australia and New Zealand) (29). While predominantly hepatoblastoma and much less frequently hepatocellular carcinoma account for two thirds, this is a group with substantial heterogeneity. The full diagnostic spectrum and individual management of the various kinds of intra-abdominal masses is beyond the scope of this thesis, however, it will explore the challenges of diagnosis and management of rarer lesions and the future of diagnosis and prospects for early detection for this specialised cohort.

1.2 End stage liver disease and Liver transplantation

The first liver transplant was performed in 1963 by Starzl et al (30). Both the technique and post operative immunosuppressive regimes were drastically improved over the subsequent 30 years resulting in modern protocols. Liver transplant is the standard of care in children with irreversible, progressive, or end-stage liver disease, fulminant hepatic failure, and inborn errors of metabolism that only liver transplantation can correct (31). In Australia children listed for liver transplantation are prioritised on clinical status. The Paediatric end stage liver disease score based on bilirubin, international normalised ratio, albumin, and growth parameters is a validated scoring system for the likelihood that a child with die from their liver disease within the next 3 months and it used to assign priorities (29). In Australia and New Zealand, the wait list mortality is low at 1.4% despite poor organ donation rates. This can largely be attributed to an intention to split policy since 2002 where most donor grafts are split providing typically segment 2 and 3 of the liver for a paediatric patient and the right lobe for an adult patient. This is reflected in whole grafts making up 75% of grafts in the US

versus just 23.7% in Australia/New Zealand in the modern era of transplant. While initial reports suggested worse outcomes in split vs whole grafts, contemporary reports show comparative outcomes (29). Absolute contraindications include severe infection outside the hepatobiliary system, irreversible and/or progressive brain injury, irreversible cardiac, renal, or pulmonary disease (though combined organ transplants may be an option in some cases), primary malignancies not affecting the hepatobiliary system, metastatic hepatobiliary cancers, immunodeficiency, and HIV infection. (32). Outcomes in Australia and New Zealand are excellent for paediatrics patients with a 10-year patient survival rate of 95% (29).

1.3 Post transplantation graft limiting complications

Liver retransplantation is the only corrective long-term management for graft failure and insurmountable graft complications. While paediatric liver transplantation outcomes have dramatically improved with improvement across multiple areas including immunosuppressive regimes, surgical expertise, donor graft preservation solutions, and recipient and donor selection, 9 – 29% of recipients will need to undergo retransplantation (33). The most common indications are hepatic artery thrombosis, primary non function, rejection, and biliary complications (6).

Hepatic artery thrombosis is the most common cause for graft failure in the immediate post operative phase accounting for 40% of retransplants (34). Presentations vary with acute severe failure, gram negative sepsis and early bile duct necrosis / biliary leak due to biliary perfusion being exclusively from the hepatic artery. If diagnosed within 24 hours of onset, urgent angiogram and operative thrombectomy may be successful however urgent retransplantation is usually required (35). Rarely, there is primary failure of the graft to function despite normal vascular flow with rates in adults reported between 1.4 to 8.4%. This is seen with failure of bile flow, acidosis, coagulopathy, and hypoglycaemia in the first 48 hours post-transplant. While it is believed to be related to graft quality the mechanism is largely unknown (36). Urgent retransplantation is always required. Both

portal vein thrombosis and hepatic vein obstruction can lead to chronic dysfunction presenting with portal hypertension and Budd Chiari syndrome respectively, however rarely require urgent retransplantation.

Rejection is the most common post-transplant complication and can occur at any post-transplant stage. The risk is highest in the first 6 months however there is an estimated 10% risk of rejection at any point in time. Most cases are due to acute cellular rejection caused by recipient T cells responding to donor alloantigen's, however rejection can also be antibody mediated. Presentation classically is with cholestasis, transaminase elevation, fever, and neutropenia, however, there are no specific reliable features. Biopsy is required for histological confirmation and to rule out rejection mimics such as CMV, adenovirus and HHV6 infection. CMV infection is associated with a 4-fold increased risk of rejection and is particularly important to recognise and treat appropriately. Treatment of acute rejection is with pulsed methylprednisolone and optimisation of calcineurin inhibitors. Adjunctive immunosuppression with MTOR Inhibitors or on rare occasions treatment with anti-thymocyte globulin, Intravenous immunoglobulins +/- plasmapheresis for refractory or antibody mediated rejection are occasionally used ((37)(38)(39)(40)). Recurrent episodes of acute rejection, development of donor specific antibodies and poor immunosuppression compliance can result in the development of chronic rejection in 3% - 17%(41). Presentation is classically with increase in bilirubin, liver enzymes with poor or gradually failing graft function. Ductopenia is seen on histology. Treatment is challenging and consists of increased target immunosuppressive levels and addition of adjunctive immunosuppressants. Data is limited however retransplantation is often required with rates variable between studies, 43.4 – 60% (42).

A 2020 retrospective cohort analysis of Australian and New Zealand liver transplant registry data demonstrated for children retransplanted between 2001 and 2017 actuarial graft survival of 84%, 75%, 70%, and 54% and patient survival of 89%, 87%, 87%, and 71% at 1 year, 5 years, 10

years, and 15 years, respectively. This was similar to the pooled survival rates for all Australasian children who underwent transplantation during that period, with actuarial survival rates of 94%, 89%, 88%, and 83% at 1 year, 5 years, 10 years, and 15 years, respectively. (6). Inevitable with improving outcomes is the fact that a growing cohort of patients are requiring a third transplant and beyond raising concerns regarding specific challenges in these patients as well as the ethical considerations of graft allocation. This thesis will explore data on outcomes and discuss current recommendations.

1.4 Intestinal transplantation, outcomes, and Morbidity

With improvements in line care and total parental nutrition (TPN) over time the rates of intestinal transplantation following a peak in 2008 have steadily fallen. It does however remain the only curative treatment for children with irreversible intestinal failure and complications of TPN. Current listing criteria include advanced or progressive intestinal failure associated liver disease, loss of vascular access, recurrent sepsis and in specific cases of invasive intra-abdominal mass.

Compared to liver transplantation there is a much higher rate of complications and morbidity following intestinal transplantation. The gut is a highly immunogenic organ and the graft contains a large amount of donor lymphoid tissue. Higher doses of immunosuppression are required and post-transplant infections, rejection, graft versus host and post-transplant lymphoproliferative disease are much more prevalent. Remarkable progress has been made with improvements in immunosuppression regimes, surgical procedure, and development of dedicated multidisciplinary teams with current graft and patient one year and five-year survival rates of 66.1 / 72.7% and 47.8 / 57.2% respectively with 90% of patients regaining enteral independence (43).

Acute rejection is the leading cause of mortality and morbidity in this group affecting 50-75% of patients (9). Rapid detection and prompt treatment is important for graft longevity, however, the only way to make the diagnosis and monitor treatment response is histopathology of graft biopsies requiring general anaesthesia and endoscopy to obtain. This is particularly problematic in children and adjunctive diagnostic and monitoring tools are required. This thesis will explore advances in this area with specific focus on the potential of point of care intestinal ultrasound as a diagnostic and monitoring modality.

1.5 Pancreatic auto islet transplantation

The standard management of pancreatitis is supportive. The North American Society for Paediatric Gastroenterology, Hepatology and Nutrition's Pancreas committee consensus recommends for acute pancreatitis fluid support with crystalloids and adequate analgesia (44). For most children with one off episodes, infrequent episodes, or with a modifiable underlying cause, this is all that is required. The management of children with frequent or more severe episodes or those who develop complications of pancreatitis is more complicated. In this group frequent disruptions to schooling and daily life from acute episodes, or from the development of chronic symptoms can greatly reduce quality of life and cause opioid dependence. This is particularly pertinent in areas such as South Australia which provides care for children in the Northern territory with a high First Nations indigenous population. This group is at particular risk with a nearly 70x prevalence of hereditary pancreatitis (1.1 / 100,000 population non-Indigenous vs 71 / 100,000 population Indigenous). PRSS1 and SPINK1 mutations have a high prevalence in this group (45).

First pioneered at the University of Minnesota, total pancreatectomy, and islet auto transplantation (TP-IAT) is a consideration for children with chronic pancreatitis without a reversible cause, chronic narcotic dependence, impaired quality of life and adequate islet cell function (Table 1). Typically, the procedure involves resection of the entire pancreas, duodenum, distal common bile duct, and often

the spleen with a view to preserve pancreatic perfusion as long as possible intraoperatively to minimize warm ischemia time to the islet cells. Following resection, the body and tail of the pancreas are sent to an islet processing lab for islet cell isolation. Once completed the islets are returned to the operating room for infusion via the portal vein allowing embolization of the islets to the liver (46). An appendix of this thesis will explore the South Australian experience with TP-IAT including advancements, particularly in post operative management, to achieve better long-term outcomes.

1.6 Structure of the Thesis

This thesis is divided into two main sections, the contextual statement detailing the story of the research and the selected manuscripts. Within the area Paediatric Hepatology, Intestinal and Pancreatic Transplantation this thesis explores Novel Aetiologies, Areas of Need and Further Research Within the Specialty and New Management Strategies.

The remainder of this chapter will continue as the contextual statement discussing the inter-relationship between the included publications, originality and authorship and the underlying field of knowledge within the current literature.

Chapter 2 contains the collection of publications published prior to commencement of this candidature. Three papers within section 1 detailing previous unrecognised novel disease processes or rare processes within Paediatric Hepatology, two papers within section 2 detailing a case of DILI and Biliary disease to highlight areas of ongoing need and research and three papers within section 3 detailing advancements in liver and intestinal transplantation. Two Appendices are included detailing work on Neonatal liver failure and Pancreatic Auto-Islet transplantation supplementing sections 2 and 3 respectively.

Chapter 3 details limitations of the included works, future research directions and concluding remarks. While part of the contextual statement a separate chapter has been provided for overall narrative flow.

2. REVIEW OF INCLUDED PAPERS

2.1 Aims of the Underpinning Publications and Relationship between Articles

The seven publications included in this thesis are divided into three sections within chapter 2, based on their aims and themes. These are Novel Aetiologies, Areas of Need and Further Research Within the Specialty, and New Management Strategies. This section explores the aims of the individual papers, as well as their relationship to each other within their sections and to the thesis overall. As a body of work the papers in this thesis explore advancements in knowledge, areas of need requiring more research, and emerging management strategies in the field of Paediatric liver, pancreatic and intestinal disease with a focus on transplantation.

2.1.1 Novel Aetiologies

Section 1 (Page 74) details three papers all of which aim to explore previously unrecognised novel disease processes or rare processes within Paediatric Hepatology with limited previous description in the literature. All three papers are written with the view to provide guidance on either or both the diagnosis and management of these conditions.

Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med.2022 aims to describe the clinical presentation, course of illness, and early outcomes of acute hepatitis of unknown cause in children following the onset of a global pandemic in 2022. The results of this study informed the subsequent national UK guidelines for management of this form of acute hepatitis as well as driving further research on the underlying aetiology. *Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023* reports two cases of children with GLIS3 mutations with chronic liver disease who required an allogenic liver transplant and presents a review to discuss the pathogenic mechanisms and liver histology resulting in their liver disease. It details management considerations unique to this

condition to ensure optimal outcomes. *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr. 2021* describes the experiences of a single centre with paediatric gastrointestinal Inflammatory myofibroblastic tumours to identify presenting features, aetiologies, prognostic indicators, and short- and long-term outcomes.

While covering a wide range of diagnostic entities these three papers all explore novel or rare diagnostic entities with limited literature available to assist the Paediatric Gastroenterologist in making informed management decisions. They all demonstrate an advancement in knowledge and understanding within Paediatric Hepatology and Transplantation as well as identifying further areas of need for each condition and avenues of research. Two papers directly describe patients who required liver transplantation as well as the unique transplant considerations for these specific conditions. While none of the patients detailed in *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr.* 2021 underwent transplantation it is a consideration with myofibroblastic tumours, with liver masses accounting for 4% of paediatric liver transplants in Australia and New Zealand (47)

All papers discuss management options based on the patients described in each cohort. *Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med*. *2022* was pivotal in informing early guidelines during a hepatitis pandemic with significant morbidity and mortality. *Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr.* 2023 details the unique transplant considerations of these patients given the complexity of concurrent renal disease, diabetes and increased long term immunosuppression risk. *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr*. 2021 discusses the wide range of management options detailed in the literature for this condition to provide practical guidance.

Finally, while these papers predominantly focus on describing novel or rare entities, all highlight areas of need and further direction within the field. *Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med.*2023 and *Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr 2023.* both detail plausible underlying aetiological processes based on clinical and histological observations within their cohorts. Particularly for the former paper this was crucial for further research to understand this process and guide disease specific management as further explored in field of knowledge within the current literature (Chapter 1, Section 3.1.1, page 43). *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr. 2021* similarly explores underlying aetiological factors as well as previously described immunohistology features that have been associated with poorer outcomes in prior studies; associations that were not able to be detected in this cohort despite being one of the largest published paediatric experiences. These themes are in turn connected to those further explored in section 2 and 3 below which detail papers describing major areas of need within the specialty, as well as novel management strategies that have emerged through prior research.

2.1.2 Areas of Need and Further Research Within the Specialty

Section 2 (Page 127) details two papers with one further paper provided as an Appendix (Appendix 1.1, page 185) with the unifying aim to explore major areas of need within the specialty. The areas explored are Drug induced liver injury (DILI), Biliary disease and Neonatal liver failure (NLF). All three papers aim to provide guidance for management of these challenging entities or add to the literature to provide new insight.

Periportal necrosis and successful liver transplantation following Lamotrigine drug induced liver injury in a child. BMJ case reports 2023 describes severe idiosyncratic liver injury secondary to Lamotrigine and explores the histological findings and specific transplant considerations. *Hepatobiliary and*

Pancreatic: A rare peribiliary lesion. J Gastroenterol Hepatol. 2021 describes a case of Eosinophilic Cholangitis with accompanying histology and radiological findings and discusses management.

Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics. 2024 is included in the Appendix. It aims to evaluate trends in etiology and outcomes of neonatal liver failure over 30 years retrospectively at a single institution. Through this data it provides guidance on outcomes as well appropriateness of liver transplantation for differing aetiological groups.

DILI, Biliary disease and NLF remain challenging areas within the specialty both due to the difficulties in researching these entities and lack of meaningful disease altering therapy. DILI and NLF are heterogenous groups greatly hindering research. Both the associated papers related to this section add to the literature by detailing specific subsets of these groups and providing guidance in these specific clinical circumstances. The aetiological processes behind paediatric biliary disease remain unknown or poorly understood. As discussed in the associated field of knowledge within the current literature section (Chapter 1, Section 3.2.2, page 61) there are likely multiple associated factors suggesting multifactorial or heterogenous aetiologies. All require further research to improve the disease course. In the case of NLF, mitochondrial, hemophagocytic lymphohistiocytosis and infectious causes, particularly Herpes Simplex Virus, have very poor outcomes and remain areas of need within this cohort. As such the sophisticated clinical documentation in these three papers is vital both to provide guidance to the clinical gastroenterologist for rare clinical presentations, and to advance the understanding of these conditions.

As a result of the lack of disease altering therapy for these conditions, despite the wide range of aetiological processes described, transplantation often remains the sole treatment option for progressive disease with three of the papers directly discussing transplant considerations. This section therefore presents unique transplant considerations for the respective cohorts, particularly in *Periportal necrosis and successful liver transplantation following Lamotrigine drug induced liver injury*

in a child. BMJ case reports and *Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics 2024*.

2.1.3 New and Emerging Management Strategies

Section 3 (Page 146) details two papers with one further paper provided as an Appendix (Appendix 1.2, Page 198) with the overall aim to explore emerging and developing transplantation strategies in Paediatric Gastroenterology.

Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation. Pediatr Transplantat 2023 aims to explore the use of point of care intestinal ultrasound for monitoring of episodes of acute cellular rejection in children post intestinal transplantation. *Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021* aims to review demographics, prognosis, and outcomes of nine children undergoing a third liver transplantation.

South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1-associated hereditary pancreatitis. Med J Aust 2021. is included in the Appendix. It aims to describe the South Australian experience with islet auto transplantation.

All three of these papers describe improvements or innovations in transplantation strategies. For the conditions and areas of need described in Sections 1 and 2 above, the end management step for progressive disease is transplantation. Outcomes for transplantation in Paediatric Gastroenterology have steadily improved over time predominantly due to improvements in surgical technique and immunosuppression regimes. However, there are ongoing areas of need for which these three papers detail advancements. Acute cellular rejection is a frequent complication post intestinal transplantation and the major cause of morbidity and mortality. Regular biopsies to obtain histology is gold standard, however, it is a source of morbidity particularly in children. Graft failure remains a

post-transplant complication for which retransplantation is the only option. While this has been established as appropriate for an initial retransplantation there is a growing number of children who require further ongoing transplants. This group has specific needs and considerations.

South Australia has a large population with hereditary pancreatitis making this an area of need specific to the authors' practice location.

Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021 and *South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1-associated hereditary pancreatitis. Med J Aust. 2021* provide specific guidance for rarer clinical presentations for the Paediatric Gastroenterologist.

The three sections of this thesis demonstrate the direction of research in paediatric hepatology and transplantation. These conditions are rare or heterogenous making research challenging and requiring reporting of cases to build a body of knowledge. Larger cohorts are able to be established and studied with time identifying areas of need. This in turn drives further research to understand underlying aetiologies to develop disease specific treatment options and management innovations.

2.2 Originality, Authorship and Contributions

Below I discuss the 9 presented publications detailing authorship, my contribution to publication, impact, originality, and impact to the published literature. Metrics specific to the publication and the journal in which it was published are included to provide quantitative evidence regarding the exposure of each article as provided by the Scopus Metrics and Google Scholar.

Of the 9 publications in this thesis, 6 are first author publications. In two of the publications (Chapter 2 - 1.1 and Appendix 1.2) the author position is second author and in one publication (Appendix 1.1) the author position is third author. Specific details of authorship and formal documentation of my

contributions to each manuscript are presented according to the CRediT contributor role taxonomy (Appendix – Table 2, page 210) ((48).

All published works in this thesis are fully original with no portions previously published elsewhere and the data gathered, analysed, and reported not previously used in any preceding publications or literature.

Publication 1.1 (Page 74)

Kelgeri C, Couper M, Gupte GL, Brant A, Patel M, Johansen L, Valamparampil J, Ong E, Hartog H, Perera MTPR, Mirza D, van Mourik I, Sharif K, Hartley J. Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med. 2022 Aug 18;387(7):611-619. doi: 10.1056/NEJMoa2206704. Epub 2022 Jul 13. PMID: 35830627.

Originality and Impact –The first cohort study investigating a high morbidity pandemic (3000 children affected worldwide, 86% native liver in situ recovery) leading to initial and urgent management recommendations and the recognition of the disease's association with human adenovirus. The publication was further highlighted in an accompanying editorial in the same issue of N Engl J Med (Karpen S N Engl J Med. 2022 Aug 18).

This paper was highly impactful. Its results guided the drafting of the national UK guidelines for management of this condition. Additionally, it has received numerous citations as detailed below including and resulting in a highly impactful study in Nature (Genomic investigations of unexplained acute hepatitis in children. Nature. 2023 Mar 30. doi: 10.1038/s41586-023-06003-w. PMID: 36996872.) which was able to delineate the underlying cause of this outbreak based on metagenomic investigations. This insight has completely changed the understanding and treatment paradigms for this condition.

Metrics for journal and for publication –

Journal Rank 2/636, 99th percentile, 1st Quartile (General Medicine), Impact factor 96.2, Cite score 145.4

Citations= 95 (Google Scholar – January 2025)

Authorship – Second author

CRediT Contribution – Additionally see letter of contribution, Appendix letters (Page - 218)

Conceptualization – Initial study design and implementation of study.

Validation – Checking data and figures for accuracy and reproducibility.

Formal analysis – Interpretation of data and generation of figures.

Investigation – Data gathering and acquisition.

Writing – original draft – Literature review and writing of introduction and discussion.

Writing – review and editing – Review and editing for mistakes, brevity, grammar. Subsequent edits as requested by reviewers and editorial office.

Publication 1.2 (Page 97)

Couper MR, Brown RM, Gupte G, Perera MTPR, Kelgeri C. Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023 Jul 1;77(1):110-114. doi: 10.1097/MPG.0000000000003773. Epub 2023 Mar 14. PMID: 36917836.

Originality and Impact – The first and only paper describing the management of liver complications in this unique condition and the only paper describing liver transplant and the unique pre and post operative management in these children. This is also the only paper to discuss and hypotheses the

underlying pathogenesis for this form of liver disease based on histological observations allowing classification as a nonsyndromic cause of bile duct paucity.

This is a niche topic however this paper has been cited in studies exploring the mechanism in which environmental toxins (2,3,7,8 Tetrachlorodibenzo-p-dioxin) disrupt hepatic function leading to steatotic liver disease in which GLIS3 genes were found to be induced suggesting a potentially wider contribution to liver disease pathogenesis outside of the mutations described.

Metrics for journal and for publication –

Journal Rank 54/330, 83rd percentile, 1st Quartile (Pediatrics, Perinatology and Child Heath), Impact factor 2.4, Cite score 5.3

Citations= 1 (Google Scholar – January 2025)

Authorship – First author

CRediT Contribution –

Methodology – Initial project planning and literature review to determine current knowledge

Validation – Checking data and figures for accuracy and reproducibility.

Investigation – All data gathering, research and data acquisition

Writing – original draft – Complete responsibility

Visualization – Preparation of manuscript for publication. Publication selected to be highlighted with infographics, work on infographics for concurrent publication.

Publication 1.3 (Page 113)

Couper MR, Eldredge JA, Kirby M, Kirby C, Moore D, Hammond P, Manton N, Glynn A, Couper RT. Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single

Centre Experience. J Pediatr Gastroenterol Nutr. 2022 Feb 1;74(2):253-257. doi: 10.1097/MPG.0000000000003311. PMID: 34596604.

Originality and Impact – The work represents the largest single centre report on this condition, providing diagnostic and management insights for the Paediatric Gastroenterologist and Histopathologist. It has subsequently been cited 5 times including a systemic review. This has allowed establishment of current advice on treatment. It has also led to research in to development and validation of a nomogram for survival prediction in patients with malignant myofibroblastic tumours.

Metrics for journal and for publication –

Journal Rank 54/330, 83rd percentile, 1st Quartile (Pediatrics, Perinatology and Child Heath), Impact factor 2.4, Cite score 5.3

Citations= 5 (Google Scholar – January 2025)

Authorship – First author

CRediT Contribution –

Methodology – Initial project planning and literature review to determine current knowledge.

Validation – Checking data and figures for accuracy and reproducibility.

Data curation – Maintaining data set for initial use and later re-use if required.

Investigation – Majority of data collection and research.

Writing – original draft – Majority of first manuscript draft and revisions.

Visualization – Article design and proofing in conjunction with editorial office.

Publication 2.1 (Page 127)

Couper MR, Brown RM, Nath S, Parida A, Kelgeri C. Periportal necrosis and successful liver transplantation following Lamotrigine drug-induced liver injury in a child. BMJ Case Rep. 2023 Nov 24;16(11):e255787. doi: 10.1136/bcr-2023-255787. PMID: 38000812; PMCID: PMC10679976.

Originality and Impact – The manuscript describes the pathogenesis of a novel drug induced liver injury (DILI) with unique histological findings. The manuscript was accepted to BMJ case reports due to the common use of Lamotrigine in children as well as the severity of the reaction resulting in the first described liver transplant in a child for Lamotrigine drug induced liver injury. The nature and histology of this DILI had not previously been reported in the literature. It has been cited in the side effects of drugs annual, an annual publication reviewing reports of notable new adverse effects and toxicities associated with medications.

Metrics for journal and for publication –

Journal Rank 292/636, 54th centile, 4th Quartile (General Medicine), Cite score 1.4

Citations= 1 (Google Scholar – January 2025)

Authorship – First author

CRediT Contribution –

Conceptualization – Joint conceptualisation with fellowship supervisor.

Methodology – Design and direction planning.

Validation - Checking data and figures for accuracy and reproducibility.

Investigation – All data gathering with assistance from histopathology unit for images and interpretation.

Writing – first draft – Completely written by myself and revisions with senior author.

Visualization – Preparation of study images with assistance from Histopathology unit. Preparation of manuscript in conjunction with journal editing department.

Publication 2.2 (Page 139)

Couper MR, Chennapragada M, Magoffin A. Hepatobiliary and Pancreatic: A rare peribiliary lesion. J Gastroenterol Hepatol. 2021 Sep;36(9):2336. doi: 10.1111/jgh.15556. Epub 2021 Jun 29. PMID: 34189779.

Originality and Impact – This short article aims to provide insight on the condition for the clinical gastroenterologist. It is novel in its detailed description of diagnosis and findings and clinically relevant due to the importance of correct diagnosis to avoid unnecessary high morbidity operative management. It is one of very few papers detailing the early investigative findings (blood results) as well as the distinctive radiological and histological findings in a child.

Metrics for journal and for publication –

Journal Rank 22/167, 87th percentile, 1st Quartile (Gastroenterology), Impact factor 3.7, Cite score 7.9

A related abstract was selected for presentation (MC) and poster at the GESA Australian Gastroenterology week 2021.

Citations= 0 (Google Scholar – January 2025)

Authorship – First author

CRediT Contribution –

Conceptualization – Study conceptualized by myself with input from fellowship supervisor

Methodology – Design and direction planning.

Investigation – All data gathering with assistance from radiology unit for images and interpretation.

Writing – original draft – Fully written by myself.

Visualization – Figure design with assistance from Radiology. Preparation of manuscript in conjunction with journal editing department.

Publication 3.1 (Page 146)

Couper MR, Valamparampil J, Thyagarajan M, Hartley J, Gupte G. Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation. Pediatr Transplant. 2023 Sep;27(6):e14574. doi: 10.1111/petr.14574. Epub 2023 Jul 17. PMID: 37458363.

Originality and Impact – The use of bedside intestinal ultrasound is well delineated in diagnosis and monitoring of inflammatory bowel disease. Its use for monitoring in intestinal transplantation is completely novel and unreported prior to this pilot study. This study is relatively new, however, provides cutoff monitoring values based on robust prospective data opening the window to future larger cohort research in this area.

Metrics for journal and for publication –

Journal Rank 147/330, 55th percentile, 2nd Quartile (Pediatrics, Perinatology and Child Health), Impact factor 1.5, Cite score 2.9.

Citations= 0 (Google Scholar – January 2025)

A related poster was presented (MC) at the Congress of the International Intestinal Rehabilitation and Transplantation Association conference 2023

Authorship – First author

CRediT Contribution –

Conceptualization –The study was fully conceptualised by myself with discussion with the broader transplant unit. The study extended my interest and post graduate qualification in pediatric intestinal ultrasonography – a relatively new tool in diagnostics and monitoring.

Methodology – I initiated and developed the study design and ethics approval myself with input from my fellowship supervisor and broader transplant team for feasibility guidance.

Software – Adoption of available ultrasound software available to perform all scans and acquire appropriate quantitative measurements.

Validation - Review and checking data and figures for reproducibility

Formal analysis – Full analysis of data by myself

Investigation – All data gathering performed by myself. All ultrasound studies performed by myself (Accredited by Gastroenterology Network for Intestinal Ultrasonography and International Bowel Ultrasound Group).

Data curation – Ongoing by myself.

Writing – original draft written completely by myself.

Visualization – Capture and preparation of ultrasound images by myself. Preparation of manuscript in conjunction with journal editing department.

Project administration – Coordination with ward and theatre staff to facilitate data acquisition and patient recruitment.

Publication 3.2 (Page 162)

Couper MR, Shun A, Siew S, O'Loughlin E, Thomas G, Andersen B, Jermyn V, Sawyer J, Stormon MO. Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021 Dec;25(8):e14092. doi: 10.1111/petr.14092. Epub 2021 Jul 27. PMID: 34313365.

Originality and Impact – The first and largest paper describing outcomes in children undergoing a third liver transplant. This paper provides guidance on a cohort of patients unavailable prior to its publication. It demonstrates comparable outcomes compared to children undergoing first and second transplant justifying third transplant from both a clinical and, in the context of limited donor organs, ethical standpoint. Additionally, this paper highlights unique considerations for management and follow up for this patient cohort as well as identifies areas of ongoing need for which further research and innovation is required.

Despite being a small cohort of patients, this paper has been cited four times including in follow up studies from centres in Germany confirming good outcomes (49). Additionally, it has been cited in a paper exploring modifiable factors influencing neurodevelopmental outcomes, an ongoing area of need for this field (50).

Metrics for journal and for publication –

Journal Rank 147/330, 55th percentile, 2nd Quartile (Pediatrics, Perinatology and Child Health), Impact factor 1.5, Cite score 2.9

Citations= 5 (Google Scholar – January 2025)

A related presentation and poster was presented (MC) at the international liver transplant society, Annual Congress 2022 and at GESA Australian Gastroenterology week 2021.

Authorship – First author

CRediT Contribution –

Methodology – Design and direction planning in conjunction with the Liver unit.

Validation - Checking data and figures for accuracy and reproducibility.

Formal analysis – Primary analysis of data with assistance from a statistician for generation of more complex analysis such as a Kaplan-Meier survival graphs.

Investigation – All data gathering and research.

Data curation – Data initially maintained by myself before handing over to the Birmingham Liver unit's nurse practitioner team at the end of my fellowship.

Writing – original draft - Fully written by myself.

Visualization – Majority of graphs and images generated by myself. Preparation of manuscript in conjunction with journal editing department.

Appendix 1.1 (Page 185)

Kelgeri C, Kanthimathinathan HK, Couper M, Alnagar A, Biradar V, Sharif K, Hartley J, Mirza D, Gupte GL. Etiology, Characteristics, and Outcomes of Neonatal Liver Failure: Lessons Learned Over the Last 3 Decades. J Pediatr. 2024 Dec;275:114245. doi: 10.1016/j.jpeds.2024.114245. Epub 2024 Aug 14. PMID: 39151605.

Originality and Impact – The cohort represents one of the largest cohort studies globally with inclusion of patients across three decades within one international paediatric transplant centre covering the northern United Kingdom, Ireland, and Scotland. This provided a unique opportunity to have adequate experience to accurately determine trends without confounding due to differences in referral base and clinical practice between centres that may be encountered in multicentre studies. This paper is recent and covers a niche area of clinical practice. As such it has not generated citations however has been utilised by Birmingham Hospital to help inform its clinical practice in this vulnerable patient group.

Metrics for journal and for publication –

Journal Rank 46/330, 86% percentile, 1st Quartile (Paediatrics, Perinatology and Child Health), Impact factor 4.41, Cite score 6.0.

Citations= 0 (Google Scholar – January 2025)

This paper was presented (MC) at the GESA Australian Gastroenterology Week September 2024.

Authorship – Third author

CRediT Contribution – Additionally see letter in support of inclusion, Appendix letters (Page - 219)

Validation – Calculation and checking of statistics to ensure accuracy.

Formal analysis – Statistical analysis of data as well as evaluation for trends.

Writing – review and editing – Main source of editing, Grammar and editing for brevity. Rewrites of introduction, results and discussion based on journal recommendations.

Appendix 1.2 (Page 198)

Eldredge J, Couper MR, Moore DJ, Khurana S, Chen JW, Couper JJ, Drogemuller CJ, Radford T, Kay TW, Loudovaris T, Wilks M, Coates PT, Couper RT. South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1-associated hereditary pancreatitis. Med J Aust. 2021 Oct 4;215(7):294-296.e1. doi: 10.5694/mja2.51247. Epub 2021 Sep 6. PMID: 34490631.

Originality and Impact – This is the only work providing insight into the Australian experience with pancreatic islet cell autotransplantation in the centre with the most experience. Of particular relevance is the high incidence of hereditary pancreatitis in Australian first nations children and the findings that the outcomes were comparable with the original international centre in Minnesota, US. This demonstrates that its use in a smaller centre and population, particularly with the establishment

of dedicated referral centres, is appropriate, something which had not been prior confirmed. It also demonstrated the practicality of pancreas transport for islet separation from Adelaide to Melbourne (800 km apart) with internationally comparative results.

This study has been cited in a follow up article in the Medical Journal of Australia exploring the clinical and genetic features of hereditary pancreatitis in South Australia.

Metrics for journal and for publication –

Journal Rank 42/636, 93rd percentile, 1st Quartile (General Medicine), Impact factor 11.4, Cite score 9.4.

Citations= 1 (Google Scholar – January 2025)

A related abstract was selected as an Oral Presentation (JE) at the Australian Gastroenterology Week September 2020.

Authorship – Second author

CRediT Contribution – Additionally see letter in support of inclusion, Appendix letters (Page - 220)

Methodology – Co-designed the report with first author

Validation – Review and checking data and figures for reproducibility

Investigation – Joint data gathering with first author (JE)

Data curation – Data curation in conjunction with first author until end of fellowship.

Writing – review and editing – Assistance with first draft and subsequent review and editing work.

3. FIELD OF KNOWLEDGE WITHIN THE CURRENT LITERATURE

3.1 Novel Aetiologies

3.1.1. New diagnostic aetiologies post the COVID pandemic

The paper *Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med.2022* (Chapter 2 - 1.1 page 74) details in retrospect the experiences of the Birmingham Children's Hospital liver unit from January 1st to April 11th 2022 with children under 6 years of age affected by a pandemic of acute hepatitis of unknown cause. It aims to describe the clinical presentation, course of illness and early outcomes of acute hepatitis of unknown cause in children. It links with the other papers within chapter 2, section 1 by discussing a novel diagnostic entity with a presumed infectious / autoimmune aetiology. Further studies stemming from this paper have contributed to our understanding of the pathogenesis in this condition and possibly to other fulminant infectious or environmental liver disease. It also explores an area of acute need given the severity of the condition with high rates of both liver failure and acute listing under the United Kingdom's super-urgent liver transplantation category.

Cases described were 4 years median age, Caucasian, previously well with no past medical history and with no geographical clustering. Cases typically had a median 3 days prodromal phase with typically vomiting and diarrhoea before developing jaundice and hepatitis. Onset was rapid with onset to peak serum bilirubin levels, alanine transaminase and Prothrombin time / International normalised ratios being a median of 5, 3 and 4 days, respectively. Patients who progressed to liver failure did so on average by day 7 (range 5 – 9 days). Failure was typically fulminant with most patients developing encephalopathy and requiring intensive care support (83%). All patients who proceeded to liver failure (13.6%) required liver transplantation. Extensive diagnostic testing was performed with 93% and 56% testing positive for Human adenovirus and Enterovirus on whole blood

PCR respectively. Liver histology where available demonstrated no chronicity, no viral inclusions and massive necrosis.

An underlying cause for acute hepatitis is often challenging to delineate. Previous large series of liver failure have found the most common diagnosis was “indeterminate” (49%) (51). Determining an underlying cause has profound implications for determining appropriate therapeutic options for these patients. Several theories have emerged regarding the underlying aetiology of the pandemic. The strong correlation with Human Adenovirus 41F made for a compelling case for this to be the underlying infectious agent. Adenovirus is a previously well recognised cause for acute hepatitis in children. This correlates well with the finding of an average higher viral load being seen in patients who progressed to liver failure. In addition, the 41F subtype is known to cause gastroenteritis in line with the prodromal phase seen in children in our study. Adenovirus 41F however previously has near exclusively been reported in immunosuppressed children and neonates. In a case series of 57 paediatric cases from 2014 the entire cohort was either post transplantation, immunosuppressed or in the neonatal age range. The majority (48%) were in patients post liver transplantation (24). All cases in our study were under 6 years of age and previously fit and well despite the unit providing care to predominantly children post both liver and intestinal transplantation. Histology also did not demonstrate any evidence of viral inclusions or adenovirus-mediated tissue damage. Finally, the pandemic was reported across 33 countries which was not consistent with a sudden outbreak of a new Adenovirus strain (2).

The timing of this pandemic occurring two years three months following the SARS-COV-2 pandemic during a period of relaxation of social distancing in effected countries is interesting. It has naturally been questioned whether SARS-COV-2 could have contributed to the acute hepatitis of unknown cause pandemic either directly or indirectly. Superantigen-mediated immune cell activation has been postulated as a process related to SARS-CoV-2 in a host primed by human adenovirus ((52), (53)). In

theory this could trigger a hyperinflammatory response in line with the massive necrosis with lack of viral inclusion bodies seen in histology samples. It has additionally been suggested that the Peyer's patches may act as a reservoir of SARS-CoV-2 with release of a viral load due to an Adenovirus gastrointestinal infection (52).

A follow up study published in Nature, March 2023, investigated 38 cases of unexplained paediatric hepatitis from the pandemic comparing to 66 age-matched immunocompetent controls and 21 immunocompromised controls using a combination of genomic, transcriptomic, proteomic and immunohistochemical methods. This study demonstrated that in addition to Human Adenovirus, both Human Herpes Virus and Adeno-associated virus were prevalent as possible causes of unknown hepatitis from the pandemic (Adeno-associated virus 100% positive in all cases vs 0% of other hepatitis comparators). This study also demonstrated the degree of diversity and lack of a unique common ancestor between case Human Adenovirus, Human herpes virus and Adeno-associated virus suggested that they were not specific to the hepatitis outbreak and instead reflected the current viral diversity in children (54). This essentially rules out a novel strain as an underlying cause for this pandemic. Adeno-associated virus is a nonenveloped virus made up of a linear single-stranded sequence of DNA. It is a helper dependent virus and is unable to replicate on its own often remaining dormant within a host's genome until a helper virus infection activates it. Adeno-associated virus is known to replicate with the help of both Adenovirus and Herpes Simplex viruses. With no previous reported disease associations, it is a leading gene delivery vector in gene therapy research and application (25). It has been postulated that social distancing during the SARS-COV-2 pandemic could have resulted in a group of under 6-year-olds with substantially reduced immunity to both Adeno-associated virus and its helper viruses. Enteric human adenovirus is very common in children under 5 years of age, however there were very few cases during the SARS-COV-2 pandemic due to reduced social mixing and strict hygiene practices. Additionally, Adeno-associated virus antibodies are typically high at birth until 7 – 11 months of age following which immunity is usually

built through exposure with respiratory human adenoviruses. The Nature study found strong transcriptomic and proteomic data from the explant livers from cases of unexplained hepatitis to suggest significant immune dysregulation as well as infiltration of CD8+ and B cell lineages. Furthermore 92% of cases were found to have HLA-DRB1*04:01 and increased levels of the same immunoglobulin variable region peptides to suggest specific antibody involvement. Following this data, it has been suggested that in genetically predisposed individuals with low immunity to these viruses due to a combination of age and social distancing allowed excessive accumulation of Adenovirus and Adeno-associated virus DNA in the liver resulting in a profound immune mediated hepatitis (54).

The pathogenesis of this hepatitis pandemic appears to be novel and there is reason to be concerned that the SARS-COV-2 pandemic was contributory to its pathogenesis. This illustrates that the long-term impacts of SARS-COV-2 are still far from understood and understanding the relation with acute severe hepatitis is crucial to delineate the disorder and assist management during future pandemics. Rigorous reporting of cases in a clinical context initially has allowed the development of both initial prognostication and management of these patients.

The follow up genomic, transcriptomic, proteomic and immunohistochemical investigations have raised questions regarding whether an initially presumed viral aetiology may become apparent or be a consequence of immune dysregulation opening the question regarding the role of immunosuppressants and immunoregulators. During the initial phases of the pandemic and with the association with Adenovirus early protocols called for the use of Cidofovir in severe cases despite the risk of nephrotoxicity (55). This treatment paradigm has now shifted towards the use of glucocorticoids eliminating this particular risk. This underlying aetiological process and treatment paradigm may also have application outside of the Adeno-associated virus trigger seen in this pandemic. For example, the team at The Royal Children's Hospital, Melbourne described a series of

patients with acute CD8 positive hepatitis in paediatric livers which responded to glucocorticoids and in some cases immunosuppression (Azathioprine). This suggests that this mechanism of acute hepatitis with liver failure may predate the pandemic and may underly a broader range of indeterminate acute hepatitis cases (56).

3.1.2. New Genetic Diagnoses and Techniques – Rare Syndromes

The paper *Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023* (Chapter 2 - 1.2 page 97) details in retrospect the experiences of the Birmingham Children's Hospital liver unit with two children with GLIS3 mutations detailing their clinical course before and after liver transplant. It aims to describe the presenting features, delineate underlying aetiology for liver disease and management specific to these patients for the clinical gastroenterologist. It links with the other papers within chapter 2, section 1 by discussing a new genetic disease associated with liver disease – an ever-expanding area of the specialty demonstrating advancement in this field. It additionally relates to the themes and publications in chapter 2, section 2 identifying an area of need. These patients require specialised management to achieve good outcomes which this paper discusses and addresses.

The two cases described had different ethnicity and gender with one Caucasian male and one south Asian female. Both patients were diagnosed within the first year of life after presenting with cholestatic liver disease, diabetes, hypothyroidism, and pancreatic insufficiency. Both patients had cystic kidney disease however patient 1 had more widespread cystic disease with large mesenteric cysts. They had quite different disease courses. Patient 1 required work up and liver transplantation at 6 years of age and patient 2 had more rapid progression requiring work up and liver transplantation at just 17 months of age. Patient 2 had ongoing complicated management post-transplant with frequent respiratory infections, acute and subsequent chronic renal disease as well

as brittle diabetes highlighting unique challenges and management considerations in these patients. Explant histology in both patients demonstrated biliary cirrhosis and bile duct paucity. In conjunction with prominent biliary structures similar to those seen in other ductal plate malformations. This is suggestive that GLIS3 liver disease is due to abnormal bile duct development and should be a diagnostic consideration of non-syndromic causes of bile duct paucity in the liver.

Bile duct paucity is defined as a bile duct to hepatic artery ratio of less than 0.5 in an adequate liver biopsy specimen (at least 10 portal tracts present) (57). It is not an uncommon finding being present in 11% of paediatric liver biopsies and as a total cohort 50% will have progressive liver disease on follow up biopsies (58). The most common associated condition with bile duct paucity is syndromic bile duct paucity or Alagille's syndrome. Non-syndromic bile duct paucity has a broad differential diagnosis and includes metabolic, genetic, infectious, immune, inflammatory, drug induced, endocrine and idiopathic aetiologies (Table 3 – Appendix tables – Causes of Paediatric non-syndromic bile duct paucity, page 211) (57). In the absence of obvious diagnostic features or cause genetic screening should be considered early in the work up of these patients. A greater understanding of mutation significance with the sequencing of the human genome and establishment of genetic databases such as the Online mendelian inheritance of man has steadily increased the utility of both gene panels and trio exome sequences. This is particularly pertinent with increasing availability and sensitivity as well as reduced cost.

The GLIS3 protein codes for a Kruppel-like zinc finger protein on chromosome 9p24.2. As discussed within the paper it has several functions however, based on results focusing on polycystic kidney disease in GLIS3 heterozygous patients. Amongst its functions, it serves as a primary cilium-associated protein. Disruption of this function could explain liver disease. The primary cilium is a key site for proteins defective in other classic forms of liver and kidney ciliopathies such as congenital hepatic fibrosis, Caroli's syndrome, and, polycystic liver disease. GLIS3 mutation related non-

syndromic bile duct paucity in the liver is a novel condition. However, this gene product may be implicated in other non-apparently related liver diseases. A 2024 paper by G. Cholico et al. referencing this thesis's paper on GLIS3 mutations described differential gene expression in the mouse liver cell following acute 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exposure. TCDD is an organic pollutant and environmental toxin most commonly formed as an unwanted product in the burning of organic materials. It is known to disrupt hepatic function leading to steatotic liver disease like pathologies. Cholico demonstrated that TCDD resulted in differential gene expression of GLIS3 in cholangiocytes suggesting a contributing pathogenesis to this form of toxin mediated liver disease (59).

GLIS3 mutations and other genetic syndromes have an underlying pathogenesis which causes multiorgan disease. For both our patients making a decision regarding whether to proceed with an isolated liver transplantation versus a multivisceral transplant was one of the more challenging management problems. Neither received a kidney or pancreatic transplant due to neither child having renal disease severe enough at the time of transplant to warrant a kidney transplant, as well as concerns regarding longer listing wait times, a more complex procedure, and higher immunosuppressive needs, particularly for a pancreatic transplant, in an acutely unwell child. Unlike liver and intestinal transplantation which have more defined listing criteria, pancreatic transplantation is discussed on a case-by-case basis. As a rule, in the United Kingdom, it is considered in children with type 1 diabetes who are listed for kidney, liver or intestinal transplantation (60). While outcomes post pancreatic transplantation remains excellent, rates have continued to drop over recent years. Mechanical solutions with pumps while still limited by the sensitivity of continuous glucose monitoring and distribution of subcutaneous insulin continue to advance and will further reduce the burden of end organ disease in diabetes. Additionally, bioartificial encapsulated islets allowing an immune privileged environment removing the need for immunosuppression while still in early trials offer a promising alternative for the future (61). Auto-islet transplantation, an

alternative for children with early diabetes and chronic / recurrent pancreatitis is discussed in the appendix of this thesis (page 198).

While by no means limited to liver disease the challenge of increased access to genetic testing is the recognition of a wide range of rare and in some cases novel mutations of uncertain clinical significance. While this can often lead to a presumptive diagnosis this information may not immediately impact on a patient's management and prognosis will often remain uncertain. While the development of databases and reporting of mutations of uncertain significance has steadily assisted in recognising the significance of mutations the future of this area lies within understanding the function of the affected genes and understanding how a patient's underlying mutation affects this function. While still not at the point of clinical application there has been substantial progress in this area in recent years. Starting in the 1950s protein structures were first determined by investigators such as Max Perutz using X-ray crystallography, a process which involves firing X-rays at crystallized proteins and inferring protein's atomic coordinates based on the diffracted radiation. The focus has shifted over the years to utilizing artificial intelligence. While initial attempts at algorithms starting in 2006 to 2016 only corresponded 30-40% (Global distance testing average) with experimental structures, more sophisticated models (AlphaFold 2) in 2020 started achieving a > 90% correspondence. This was utilised in early 2020 to successfully predict a handful of SARS-CoV2 protein structures prior to traditional experimental methods (62). With human proteins this may allow us to determine for example receptor sites in membrane proteins or catalytic sites in enzymes. This will allow novel therapeutics to be developed. Additionally, we can predict human protein structure from the genes assisting in determining whether a genetic defect of unknown significance results in functional perturbation of the protein and subsequent pathogenicity.

This paper adds to the literature on GLIS3 and non-syndromic bile duct paucity and its management, particularly regarding transplant considerations. Current diagnosis and management of syndromic

liver disease relies on the application of basic principles with reporting of long-term outcomes remaining crucial for development of condition specific prognostics and management. The ongoing rapid development of genetic diagnostic techniques, assessment of gene function and underlying pathogenesis of conditions and transplant alternatives is the future of individualised care in this area. The delineation of rare diseases will help in delineating risk factors for multifactorial lifestyle diseases in that genes are ubiquitous and whilst genetic syndromes are rare, polymorphisms which predispose to lifestyle disease may be much more common. This is demonstrated in the potential overlap in GLIS3 involvement in both the congenital mutation cases described in this paper and as a potential pathogenesis in the hepatic disease seen in TCDD exposure. The more we know about individual diseases the better we will understand the phenotype of various liver pathologies.

3.1.3 New Genetic Diagnoses and Techniques – Cancers and screening

The paper *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr. 2021* (Chapter 2 - 1.3 page 113) reports a retrospective longitudinal cohort of all children with a diagnosis of an abdominal Inflammatory myofibroblastic tumour (IMT) and followed at the Women's and Children's Hospital, Adelaide from 2006 until October 2019. It aims to describe the lesion as well as management for the clinical gastroenterologist. It links with the other papers within chapter 2, section 1 by advancing the literature in this area, discussing, and delineating a rare form of gastrointestinal tumour that affects the liver and the bowel as well as acting as discussion for ongoing areas of need for diagnostics and management of liver lesions.

Seven patients were identified over this time period with four patients with gastrointestinal (stomach, duodenum, small intestine and multifocal abdominal), two patients with liver and one case of head of the pancreas IMT. Diagnosis was in the context of associated organ obstructive symptoms or as an incidental finding on imaging. One patient had more generalised constitutional symptoms

with elevated inflammatory markers. Diagnosis was via imaging or gastroscopy with subsequent histopathology. Where feasible full surgical resection was undertaken (3 patients) all achieving complete remission. The remaining patients received various treatment including chemotherapy, medical management (NSAIDS, dexamethasone and Imatinib) and expectant management with monitoring. All patients remained in remission or with a stable lesion with ongoing monitoring asides from an 8-year-old who presented with multifocal abdominal IMT and underwent incomplete resection. She required 2 subsequent courses of chemotherapy after initial induction due to local recurrence.

Diagnosis and management of liver and other intra-abdominal masses can be a conundrum for the Paediatric Gastroenterologist as well as the surgical pathologist. They demonstrate substantial morphologic heterogeneity. While the liver constitutes the third most common solid abdominal tumour in children, they remain comparatively rare with an incidence of 1.3% of all paediatric malignancies in children under the age of 19 years of age (63). Of these hepatoblastoma and hepatocellular carcinoma are the most common accounting for two thirds of all hepatic neoplasms. Management is complete resection where feasible with chemotherapy for unresectable lesions before definitive resection can be performed. Liver transplantation is an option in children who do not adequately respond to resection and chemotherapy, with no evidence of metastatic disease or when metastasis has been controlled. This indication makes up 4% of paediatric liver transplants in Australia and New Zealand (47). Additionally, there are a wide range of classic paediatric benign liver tumours including hepatocellular adenomas, hemangiomas, mesenchymal hamartomas, and focal nodular hyperplasia with established recommendations for monitoring and management (64).

Outside of the classical paediatric intraabdominal lesions there is a large differential list of comparatively rare lesions, including IMT. The described study has limitations. It is small and retrospective however despite these limitations studies of this nature are important. Paediatric gastrointestinal IMTs fall into the list of rare lesions encountered by the Paediatric

Gastroenterologist. With an estimated incidence of 1 in a million children and with less than 40% presenting in the abdomen, establishment of guidelines is challenging. This is seen in this study with various treatment modalities utilised based on the understanding of these lesions' pathogenesis at the time. Given these lesions' rarity, collective experience provided in smaller case reports and retrospective cohorts is important to help develop guidance in their management. This is demonstrated by the evolution of subsequent literature on this topic following publication of this paper. Systematic reviews have confirmed this papers findings that a negative tumour margin has a strongly positive outcome (Recurrence 6.8% vs 51 % and Mortality 1% vs 13.2%) (65). Similarly, treatment algorithms have been proposed with the steady accumulation of experience and a literature base. Current recommendations are for surgical resection for localized lesions without regional spread, surgery and chemotherapy (doxorubicin, vincristine and cyclophosphamide) for localised lesions with incomplete resection or regional lymph nodal spread and longer course chemotherapy (Methotrexate and Vinorelbine), radiotherapy or ALK inhibitor for distant metastases at onset (66)

A challenge in paediatric liver disease in general is diagnosis of late-stage disease leading to a poorer prognosis. The liver is an adaptive and regenerative organ with substantial functional reserve resulting in symptoms only after substantial damage. This is particularly true in liver and gastrointestinal malignancy. As seen in this study the majority presented only when mass effect resulted in obstructive symptoms. Given improved outcomes with early diagnosis, this makes screening in children appealing. However, large scale implementation is limited by comparative rarity skewing the cost – benefit ratio. There are several well-known genetic conditions such as glycogen storage disease, Hurler syndrome, Tyrosinemia, Galactosemia, Li-Fraumeni, Fanconi anaemia and Beckwith-Wiedemann syndrome which have a well-established tumour risk and require regular monitoring. Similarly, hepatitis B, previous Fontan procedure, and cirrhotic liver disease also require regular monitoring. Recent studies have demonstrated that childhood tumours have 14 times lower somatic alterations than adult tumours (67). A logical explanation is that somatic mutations are

accumulated during aging and this has raised interest in underlying predisposing germline mutations both for the purpose of screening and understanding of the genetic basis of childhood tumours. Studies have shown up to 14% of childhood cancer sufferers who underwent whole exome sequencing were carriers of alternation in cancer predisposition genes. Genome Wide Association Sequencing studies have additionally been useful for identifying single nucleotide polymorphisms in particular when identifying several that may have a cumulative cancer risk (68).

IMT do not currently fall into this group and are predominantly associated with somatic mutations with a particular association with ALK gene rearrangements. This may however be due to the challenges of studying and identifying trends in rare sporadic sarcomas. Broader studies investigating the role of known cancer-associated germline mutations in sarcomas including patients with IMT demonstrated a 10.6% prevalence (69). An advancing interest in expanded screening and reporting of variants of unknown certainty, research involving national or multicentre cancer databases and meta-analysis of individual reported cases series is crucial in this area going forward.

While still early days based on these findings there is interest in expanded newborn screening. The "Newborns in SA" study, conducted through SA Pathology and the Women's and Children's Hospital in Adelaide, South Australia, serves as a key example from the author's practice locale. This study aims to evaluate the cost-benefit of expanding newborn screening with exome sequencing to detect over 600 genetic conditions, extending beyond the scope of the standard newborn screening blood spot card as part of a collaborative of similar newborn screening studies around Australia. From a gastrointestinal perspective this will include increased cancer risk genes such as Li-Fraumeni and Mismatch repair cancer syndromes 1 – 3 amongst many others.

3.2. Areas of Need and Further Research Within the Specialty

3.2.1. Drug Induced Liver Injury

The paper *Periportal necrosis and successful liver transplantation following Lamotrigine drug induced liver injury in a child. BMJ case reports 2024* (Chapter 2 – 2.1, Page 127) details in retrospect the management of a child following a Lamotrigine drug induced liver injury (DILI) before and after transplantation at Birmingham Children's Hospital liver unit. It aims to report a high morbidity drug induced liver injury from a commonly used childhood anti-epileptic agent. This case describes key learning points from this case, it also describes the first reported liver transplant for lamotrigine induced drug injury and postulates underlying pathological mechanisms based on the liver histology. The publication reflects the theme of areas of need in paediatric liver disease and acts as a discussion on DILI. DILI despite being one of the most common forms of liver injury in children has sparse literature and few treatment options that improve outcomes outside of specific management for a handful of agent specific antidotes.

The patient initially presented with new-onset vomiting and increasing lethargy. She had a background of attention deficit hyperactivity disorder, behavioural disturbances, and self-harm. For her conditions she had been commenced on Lamotrigine three weeks prior for which the dose had been increased three days prior to presentation. She was additionally on long term Atomoxetine. Initial work up demonstrated a significant hyperammonaemia (peak = 942 umol/L), coagulopathy (INR = 5.2) and transaminitis. Aetiologic work up was unremarkable and despite supportive care she required transplantation due to acute severe hepatic failure. Transplantation was uncomplicated and she was discharged with no neurological sequelae. This study illustrates a severe DILI induced by a commonly prescribed anti-epileptic in children. Hepatic failure cases in young children with a background of or presenting with neurological symptoms are more complex and alternative diagnoses that may preclude transplant need to be excluded. Lamotrigine DILI typically presents with

a self-limiting drug reaction with eosinophilia and systemic symptoms (DRESS). This case was unique in its severity and histological findings demonstrating periportal necrosis and low numbers of inflammatory cells. Atomoxetine is also known to rarely cause hepatotoxicity through the production of a toxic intermediate which is normally extensively metabolised by CYP2D6. Lamotrigine is known to strongly inhibit CYP2D6 likely resulting in a synergistic effect due to polypharmacy (70). This report documents this unique reaction and emphasises the heterogeneity of mechanisms resulting in DILI.

There are several areas where DILI research is lacking, particularly in children. Diagnosis of DILI is by exclusion often necessitating extensive testing and liver biopsy. With common offending agents in an otherwise healthy child this is often simple however other cases such as the one detailed in this paper can be more challenging. Establishing the correct diagnosis is crucial to ensure condition specific treatment is not required as well as to ensure in more severe cases that transplantation is not contraindicated. The most common offending agents in children are paracetamol, antibiotics (particularly flucloxacillin, amoxicillin, clavulanic acid, nitrofurantoin, doxycycline and anti-Tuberculous drugs) and antiepileptics (particularly Carbamazepine and Sodium Valproate) (71). Diagnosis can be substantially more challenging with new agents or rare interactions. Research is this area is sparse and almost exclusively performed in adult patient cohorts, however advancements have been made -

Firstly, in clinical adult practice the Roussel Uclaf Causality Assessment Method (Table 4 - Roussel Uclaf Causality Assessment Method, Page 212) has been in use for the last 30 years to grade likelihood of a DILI providing high reproducibility and minimal inter-observer variability. Validation studies demonstrate an 86% sensitivity, 89% specificity, 93% positive predictive values and 78% negative predictive value (72). While the scoring system has been applied to DILI research in children it has not been clinical validated and is not in wide spread use in paediatric clinical practice.

Secondly, one of the challenges of diagnosing DILI is the lack of specific biomarkers and given the wide range of pathogenesis of DILI, a non-agent specific marker is unlikely. There has however been some progress in agent specific testing which holds promise. Genetic testing has become effective due to the associations between specific human leucocyte antigens alleles and agent specific idiosyncratic DILI. In current clinical practice this has utility in assisting diagnosis. While not diagnostic in isolation, a positive DILI associated HLA allele in conjunction with exposure to a causative drug is highly suggestive of a diagnosis. Similarly, for certain HLA alleles a negative result has a strong negative predictive value for DILI for certain agents (Table 5, Known DILI associated HLA alleles with associated drugs, Page 213) (73). While not in widespread use currently HLA allele testing holds promise for screening prior to commencement of certain DILI causative agents.

Lastly, while still in early stages, human liver hepatoid screening offers the potential of future screening for DILI risk and individualised medicine. Simultaneously this is an exciting area to allow further research into agent specific DILI pathogenesis as well as testing of new agents without the need for animal or human clinical trials (74). This area is explored further in Chapter 3.2 – Future directions (Page 174).

With some exceptions for specific agents' treatment of DILI in children is limited to ceasing the offending agent and supportive care. While generally most patients will recover uneventfully, recovery may be protracted with some insults resulting in elevated transaminases for two years or more. Individual prognostication remains challenging particularly in children. In adult practice Hy's law, named after Hyman Zimmerman, helps determine patients at a high risk of acute liver failure predominantly based on degree of transaminase rise and hyperbilirubinemia. Population studies have demonstrated a high sensitivity (92%) and negative predictive value (99%) but low sensitivity (68%) and positive predictive value (2%) (75). To date there have been no major studies investigating prognostication in children and no scoring systems are validated or in wide spread use for Paediatric

DILI with one notable exception. While most Paracetamol DILI recover with appropriate administration of N-Acetylcysteine, liver transplantation is occasionally indicated for fulminant failure. The King's College criteria for Acetaminophen toxicity is utilised to help determine patients who require referral and work up for liver transplantation with the primary reference study including children (Table 6, King's College criteria for Acetaminophen toxicity, Page 214) (76). Follow up studies, however, have raised concerns regarding sensitivity (69%) emphasising its use to guide but not determine clinical practice (4).

While none are in widespread use in Paediatric clinical practice there has been limited research into specific agents to treat DILI. Typically, these agents have limited evidence or demonstrate improvement in biochemical markers without evidence of improvement of long-term outcomes. Agents can be classified into four categories, 1) hepatoprotective drugs, 2) anticholestatic drugs, 3) immunosuppressants and 4) agent specific treatments.

1- N-Acetylcysteine (NAC) is one of the most used hepatoprotective agents with its role as an antidote in paracetamol overdose well established. While studies have suggested that NAC may reduce hospital stay for non-paracetamol DILI studies have not demonstrated any change in long term outcome. A single study in children demonstrated no benefit in children (77). Glycyrrhizin acid acts as an anti-oxidation and anti-inflammatory agent and is thought to protect the liver against inflammatory damage. It is approved for use in China to treat acute DILI with randomized double-blind studies in adults demonstrating significantly greater rates of transaminase normalization at 4 weeks post DILI (85.71% vs 61.02% control) (78). Other agents include Polyene Phosphatidylcholine which provides endogenous phospholipids with the view to repair damaged hepatocytes and organelle membranes and Bicyclol which inhibits inflammatory regulatory factors which induce liver injury as well as reactive oxygen species. Studies in both are limited and like other mentioned agents suggest a hastened normalization of transaminases but have no evidence for improvement in long

term clinical outcomes. Silymarin derived from milk thistle is also postulated to be helpful (77).

2- Certain agents, particularly those excreted by the liver into the bile, are associated with a cholestatic pattern of DILI with more severe manifestations resulting in drug-induced cholangiopathy and vanishing bile duct syndrome. There has been interest in the use of anticholestatic agents to treat this subset of DILI patients. Ursodeoxycholic acid is the most used anticholestatic agent in clinical practice with applications in primary biliary cirrhosis, primary sclerosing cholangitis and other cholestatic conditions. It reduces the cholesterol saturation index of bile and inhibits the absorption of cholesterol in the intestine. Research in DILI has been conflicting with some suggesting expediated normalization of liver enzymes while others showing no noticeable difference (77). S-Adenosylmethionine is the active form of methionine. It is involved in the detoxification of bile acid metabolism and promotes the synthesis and secretion of bile acids preventing liver cell injury and necrosis (79). Studies investigating use in both generalised DILI and specifically DILI due to immunosuppressants and cancer drugs have demonstrated significantly improved liver enzymes and bilirubin as well as a reduction in cholestatic symptoms and fatigue (77). Finally, Cholestyramine is a non-absorbable styrene anion exchange resin. It is commonly used for pruritis due to cholestasis and prevents the reabsorption of cholic acid. It has been found to have a role in treatment of DILI due to Leflunomide and Terbinafine specifically however use is limited to these specific agents exclusively (80). Drugs which reduce absorption of toxic bile metabolites (ie IBAT inhibitors, Odevixibat and Maralixibat) or drugs which act on Farnesoid X activated receptor (ie Obeticholic acid) may also in select cases have some utility however studies are lacking to date.

3- DILI often involves an inflammatory response with immune system activation and an adaptive immune attack. This often manifests with extrahepatic immune injury with fever

and cutaneous manifestations (81). This has led to interest in the role of immunosuppressant and anti-inflammatory drugs for the treatment of DILI. With their widespread anti-inflammatory and immunosuppressive effects glucocorticoids are the most frequently used agents of this class. They have been used as empiric treatment for DILI especially in cases with signs of hypersensitivity or autoimmunity, particularly drug associated lupus. The most frequent agents involved are Infliximab and Hydralazine though the later is not often used in children. Analysis of the American DILI Network Database showed that in adults glucocorticoids were used in 82% of patients who died or proceeded to transplant and 36.6% of patients who survived (82). Numerous studies have investigated the evidence for their use with variable and conflicting findings however even studies finding no benefit have deemed glucocorticoid therapy safe. A summary of the literature suggests that glucocorticoids may have a potential therapeutic effect particularly in patients with severe DILI and cholestasis despite withdrawal of offending agents and standard therapy (77).

4- In addition to the three above categories certain agents are useful for specific aetiologies of DILI playing a role as an antidote or acting specifically based on the underlying resulting pathogenesis. These include N-Acetylcysteine for Paracetamol, Silymarin for Toadstool poisoning, L-Carnitine for Valproic acid and Anticoagulants / Defibrotide for sinusoidal obstructive syndrome / hepatic veno-occlusive disease particularly following hematopoietic stem cell transplantation and certain forms of chemotherapy, particularly alkylating agents.

DILI is a common and increasing cause of liver injury in children. Research and innovation in this area has been challenging in children due to a lack of specific diagnostic and prognostic tools and the wide range of underlying mechanisms of liver injury between different inducing agents and individual patients. Further research particularly regarding underlying pathogenic mechanisms to allow individualised treatment is crucial to ongoing improvements in this area.

3.2.2. Biliary Disease

The paper *Hepatobiliary and Pancreatic: A rare peribiliary lesion. J Gastroenterol Hepatol. 2021* (Chapter 2 – 2.2, page 139) details in retrospect pertinent diagnostic features of a child with eosinophilic cholangitis who presented to and underwent treatment at The Children's Hospital at Westmead. This publication aims to raise awareness and describe a rare biliary disease – Eosinophilic cholangitis. This paper links to the other papers and themes by discussing a relatively new diagnostic entity with very specific management consideration to avoid unnecessary morbidity. Additionally biliary disease is a major area of need within paediatric hepatology. Typically, there are only supportive cares available with no disease altering management options. Disease progression often necessitates liver transplant.

The patient presented with nonspecific systemic symptoms, cholestatic symptoms and hepatomegaly. Full blood count and liver function tests demonstrated a predominantly biliary pattern of liver derangement and profound eosinophilia (30.37 x 10^9/L). Imaging demonstrated markedly dilated intrahepatic ducts converging on an ill-defined T2 hyperintense lesion infiltrating along the portal tracts towards the hilum. Histology was obtained via percutaneous transhepatic cholangiography and demonstrated a mixed infiltrate of lymphocytes, eosinophils, and neutrophils consistent with a diagnosis of eosinophilic cholangitis. The patient was treated with balloon dilatation and biliary drain insertion. Glucocorticoids were considered; however, resolution was achieved with expectant management alone and sustained following drain removal.

Eosinophilic cholangitis is a benign condition complicated by diagnostic challenges. There are 39 cases reported in the literature (83). It consists of transmural eosinophilic infiltration of the biliary tree resulting in fibrosis and stricturing. As was in the case of this patient it may present early on with

peripheral eosinophilia (66%). Its tumour-like appearance results in it commonly being misdiagnosed as a malignancy resulting in unnecessary and often extensive surgical resection (66% of cases) in part due to concerns regarding the consequences of missing and mismanaging malignant disease (83). Case reports suggest the condition is more prevalent in men with symptoms consisting of pain (63%), jaundice (53%) and fatigue. Liver function tests typically demonstrate hyperbilirubinemia (83). The pathogenesis of this condition is unknown. It has been proposed that it may be a form of cholecystitis resulting from infections due to specific organisms such as Enterobacter aerogenes or Candida albicans (84). However, it has also been noted to be more prevalent in individuals with a history of atopy (85). Yamuna et al have suggested a diagnostic pathway suggesting the diagnosis can be made in a patient with consistent MRI / CT features in conjunction with peripheral eosinophilia. In the absence of eosinophilia or if there is uncertainty, biopsy is required to look for classic histological features. Treatment in confirmed cases consists of biliary stenting as required to relieve obstruction and high dose steroids for 2 weeks with a subsequent slow taper over at least 6 months. Eosinophilic cholangitis is highly responsive to steroid therapy however 16% will develop recurrence of their disease (83).

It has been postulated that Eosinophilic cholangitis may be an early stage of Primary sclerosing cholangitis (PSC). Because it results in fibrosis of the intrahepatic and extrahepatic bile ducts, PSC is one of the most challenging conditions to manage in Paediatric Hepatology due to a complete lack of meaningful therapy. Management is supportive only with liver transplant necessitated for progressive disease (40% of cases) (86,87). A considerable number (10-20%) develop cholangiocarcinoma and there is an overall 3-fold increase in mortality rate compared to the general population (87,88). Reports from Japan have observed 27% of cases of PSC have concurrent eosinophilia and experimental studies have demonstrated a close relationship between eosinophilic infiltration and fibrosis in conditions such as scleroderma (89). Certainly, untreated Eosinophilic cholangitis results in sclerosing cholangitis and stricturing which closely resembles PSC. Similar

eosinophilic process such as Eosinophilic esophagitis are also known to lead to fibrosis and stricturing in a similar manner. Additionally Inflammatory bowel disease, which is strongly associated with PSC, asthma and non-pulmonary atopic conditions share pathophysiological similarities. Histamine and mast cell activity are similar in these conditions and IgE is a key immunoglobulin in their pathogenesis's (90). Unfortunately, patients in the early stages of Eosinophilic cholangitis, Primary sclerosing cholangitis and other biliary diseases are often asymptomatic resulting in most patients presenting with established disease. This in conjunction with a lack of any disease specific biomarkers makes study of the underlying initial aetiological process challenging.

This lack of understanding of the underlying molecular mechanisms underpinning these chronic biliary diseases, particularly PSC, greatly hinders the development of meaningful therapies. Research in this area however has provided some insights. Inflammatory bowel disease, particularly Ulcerative colitis, is a significant risk factor for the development of PSC with 60 – 80% of patients with PSC also having underlying inflammatory bowel disease (91). Additionally, it is recognised that PSC is strongly associated with HLA class II haplotypes (DRB1*03, DQA1*0501, DQB1*02, DRB1*13, DQA1*0103 and DQB1*0603) (88). Both suggest an underlining autoimmune - inflammatory process. A microbial influence has also been recognised raising a possible gut-liver axis involvement. Studies have demonstrated a pro-inflammatory microbial signature in PSC patients (reduction in beneficial short chain fatty acid producers), overall reduced bacterial diversity, and a loss of beneficial bacterially generated prebiotics (87). It has been postulated that in the context of an inflamed bowel cholangiocytes may be exposed to an increase in pathogen associated molecular patterns such as lipopolysaccharides and bacterial antigens resulting in cholangiocytes expressing toll-like receptors producing pro-inflammatory and chemotactic cytokines (92). Alternatively gut derived memory T cells may be recruited to the liver which are regulated by liver T regs. These in turn are regulated by IL-2 and IFN-gamma pathways which can be overexpressed in response to microbial associated molecular patterns resulting in hyperinflammation (87). A causative relation however is challenging

to establish due to cholestatic liver disease in itself resulting in gut dysbiosis through altered secretion of beneficial bile acids.

These hints at contributing factors have provided potential targets for therapy, some which are utilised to varying success in clinical practice. Selective antibiotics against enteric bacteria, particularly Vancomycin, has been postulated to be beneficial in select cases. This is part of standard practice for children with active Ulcerative colitis with PSC failing conventional medical therapy at Brisbane Children's Hospital, one of the authors fellowship training sites and has been adopted elsewhere. Auditing has demonstrated deep remission of patient's ulcerative colitis in 92% and improvement in liver function tests up to 12 months post a median of 6 months of oral vancomycin therapy (93). Other studies however have brought into question whether oral Vancomycin modifies long term disease outcome with no improvement in biopsy proven fibrosis stage following treatment (93) Similarly Faecal microbial transplant has been considered as a possible therapeutic option. At this stage however only one small pilot study of ten patients is available demonstrating a decrease in ALP levels in 30% of participants (94). Bacteriophage therapy and vaccines have been postulated as possible treatment modalities however have not been studied in any meaningful capacity to date. Despite its close association with Inflammatory bowel disease and compelling evidence for an immune mediated disease immunosuppressive agents including biologics have been shown to have minimal to no clinical benefit. Additionally, Vedolizumab may worsen liver biochemistry in PSC. While this report requires further research and verification, this is surprising as in theory an integrin receptor antagonist would block gut homing lymphocyte trafficking to the bile ducts which would be expected to be beneficial (95).

With the limited range of treatment options further research on the underlying aetiology to provide therapeutic targets is sorely needed. Cholangiocyte specific organoids to study underlying molecular

processes in biliary disease show promise in this area and will be further discussed in Chapter 3.2 – Future directions (Page 174).

Studies starting with case reports to increase the recognition of conditions such as Eosinophilic Cholangitis are crucial to allow proper diagnosis and to avoid unnecessary treatment resulting in morbidity. Additionally, the understanding of the underpinning molecular mechanisms of chronic biliary conditions that affect children is lacking in turn impeding the development of disease altering therapies. This is an area of substantial need are further studies and reporting are sorely needed.

3.2.3. Diagnostic Trends in Neonatal Liver Failure over Three Decades

See Appendix 1.1 page 185.

3.3. New and Emerging Management Strategies

3.3.1. Surveillance in Children Post Intestinal Transplantation

The paper *Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation. Pediatr Transplantat 2023* (Chapter 2 – 3.1, Page 146) is a prospective pilot study exploring the feasibility of monitoring children post intestinal transplantation for rejection with intestinal ultrasound. It details the ultrasound findings in context of histology results in all children undergoing a biopsy for suspected rejection or as part of routine surveillance at the Birmingham Children's Hospital, United Kingdom over a 6-month period. It aims to explore whether Intestinal Ultrasound can be utilised for non-invasive monitoring in children post intestinal transplantation. This publication ties into the themes of advancement as well as areas of need. Acute rejection in this patient group is the main source of graft failure which combined with the monitoring required to screen for it result in significant morbidity for these children.

During the study period three patients were diagnosed with biopsy proven acute rejection and a further seven patients had surveillance biopsies performed with no evidence of rejection. All three patients diagnosed with an episodes of acute rejection were demonstrated to have increased bowel wall thickness, vascularity and mesenteric inflammation on ultrasound performed on the same day as the biopsy. The increase in bowel wall thickness was predominantly due to expansion of the submucosal layer. The thickness of the submucosal layer in the patients with rejection was 3.4 – 5.1 mm. With treatment, clinical, and, histological improvement, there was a reduction in inflammatory ultrasound findings. Ultrasound studies performed on patients with no rejection on biopsy demonstrated an average bowel wall thickness of 1.54 mm (range 0.9 – 2.8 mm) suggesting a normal cutoff value of 3 mm may be appropriate in this patient group.

Acute rejection is the main cause of morbidity and reduced both patient and graft survival post intestinal transplantation (9). Of all solid organ transplants it has the highest rate of rejection (>40%) (8). Clinical and biochemical features while suggestive are nonspecific and may be absent. Establishing the diagnosis is essential to ensure appropriate treatment and resolution. As such regular surveillance in this patient cohort is imperative and currently graft biopsies for histology are the only way to diagnose or exclude rejection. This is particularly arduous and invasive in children and this is a patient cohort with a high medical burden. At the Birmingham Children's Hospital, in the immediate post-transplant stage, patients require surveillance biopsies twice a week. Frequency of surveillance decreases with time in stable patients, however at a minimum patients require once to twice yearly biopsies. Established rejection additionally requires follow up biopsies to ensure treatment response and determine whether treatment escalation is required. This can be challenging in severe rejection as these children are often clinically unstable and commonly require intensive supportive care. Additionally, there is ongoing concern regarding the long-term risks of repeat general anaesthetics in children. While a challenging area of research with inconsistent findings recent large systematic reviews continue to suggest that repeated and longer duration general anaesthetics may be associated with adverse neurocognitive effects particularly in younger children < 7 years of age (96).

Due to the high-test burden, alternative and adjunctive monitoring techniques, particularly potential biomarkers of rejection, have been actively explored. Citrulline is an amino acid predominantly derived from small bowel enterocytes and has previously been studied in short bowel syndrome as a marker of intestinal function and a predictor of nutritional outcome (97). It has been shown that citrulline levels are inversely proportional to the degree of rejection in intestinal transplant patients. A cut-off of 13 umol/L was found to have a 96 and 69% sensitivity and specificity in adults and children for moderate and severe rejection. Clinical use is hindered by a lower sensitivity for mild

rejection (70%), inability to differentiate rejection from other causes of enteritis and levels being affected by factors such as time post-surgery and renal function (98). Calprotectin is a protein found within neutrophilic cytosol and its faecal concentration increases when there is gastrointestinal mucosal infiltration of leukocytes. Faecal calprotectin testing has wide spread application in screening and monitoring inflammatory bowel disease and it stands to reason that it could be useful for monitoring other causes of enteritis such as rejection. Large cohort studies in adults have suggested a sensitivity of 76 – 100% for this application. However, significant interpatient variability has made proposing generalized cut-off values challenging. Unsurprisingly studies have found a low specificity for rejection at only 47%. Currently use is limited to surveillance in asymptomatic individuals where endoscopy is troublesome (98). Rejection is caused by cytotoxic T cells and there has been interest in whether mediators of cytotoxic T cells such as perforin and granzyme B could be good early biomarkers. Given a more specific link with the pathogenesis of rejection itself studies have shown more promising sensitivities and specificities (Granzyme 80% and 79% and Perforin 70% and 79% for sensitivity and specificity respectively) however use has not reached clinical practice and cut off values have not been clearly delineated. Additionally due to variable expression during the first month post-transplant markers were not found to be useful in this period of greatest risk nor were they useful for monitoring as typical immunosuppressive regimes decreased the concentration of both molecules regardless of clinical or histological improvement (98).

One of the limitations of this study similar to other proposed biomarkers for rejection is that it remains unclear whether ultrasound can differentiate between rejection and other causes of enteritis. While numbers were small all patients who were found to have had an acute episode of rejection were found to have a substantial expansion of the submucosal layer (range 66.6 – 76.2% of the overall bowel wall thickening). This is not the case in Inflammatory bowel disease or infectious enteritis where most of the current clinical experience in point of care intestinal ultrasound lies. The

submucosal layer contains the arterial plexus with its arterioles branching into capillary networks to supply the mucosa and muscularis layers. Typically, the predominant site of damage during acute rejection is the intestinal mucosa with exfoliative rejection resulting in the entire mucosal layer sloughing off (99). Whether this is a feature more specific for acute rejection will require further study with larger cohorts and comparison to imaging findings in other causes of enteritis post intestinal transplantation. Another potential limitation of intestinal ultrasound in this context is the potential to miss disease. Typically, enteritis from acute rejection is diffuse however particularly in mild cases patchy disease is possible with intervening segments of normal graft (99). One of the major advantages of intestinal ultrasound over monitoring with markers such as calprotectin in inflammatory bowel disease is the ability to localise and map out disease. As typical anatomy is lost during an intestinal transplant and it is not possible to track and visualise the entire small bowel there is the potential that mild and patchy disease could be missed. Larger cohorts and evaluation of patients with mild rejection is needed to explore how much this affects the modalities sensitivity.

Acute rejection post intestinal transplant is a common and a survival limiting complication. Traditional monitoring strategies while effective have significant morbidity and burden on patients and families particular in the paediatric cohort. Intestinal ultrasound shows significant promise for both diagnosis and monitoring of rejection. However, larger studies are required to further delineate limitations. Clinical application of intestinal ultrasound in this field in the first instance will be best suited to monitoring patients during episodes of rejection found to have features at the time of diagnostic biopsies.

3.3.2. Graft Failure Post-Transplant, Hepatic

The paper *Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021* (Chapter 2 – 3.2, Page 162) details in retrospect the experiences of the Children's Hospital Westmead, Syndey transplant program from its inception in 1986 until 2020 with all children who underwent a third liver transplantation. It aims to assess demographics, prognosis, and outcome measures in this growing cohort of children. This paper links with the other papers in chapter 2, section 3 discussing evolving management strategies and addresses the theme of areas of need within Paediatric Hepatology. Liver transplant, outside of cases with contraindications, remains the universal end point of management for progressive liver disease regardless of aetiology. As patient survival improves a growing number require retransplantation for graft related complications. This cohort has unique considerations and their management is a growing area of need within the specialty.

Nine patients were identified over the program's course with two patients in the 1990's, four patients in the 2000s and three patients in the 2010's onwards. Three of the nine patients passed away all within the post operative period (< 26 days post-transplant) all from sepsis. This included both patients who underwent retransplantation in the 1990s prior to the advent of modern immunosuppressive regimes and operative techniques. Third transplants had on average higher operative times, cold ischaemic times, and higher average transfusion volumes. One patient developed renal failure requiring dialysis followed by a renal transplant. Both learning difficulties and psychological issues were prevalent. When considering the modern era of transplant, following the inception of modern immunosuppressive regimes and surgical techniques, alone the survival rate was 86% matching the 5-year survival seen in children post second transplant of 87%. With a lack of alternative management strategies, low wait list mortality (1.4%) in Australasia and comparable organ and patient survival to first and second transplant, listing for a third transplant in children where indicated appears reasonable however as further discussed the neurological and psychological impacts are considerable. Subsequent studies referencing this paper collaborate this result (49)

The main limitations of this paper are a small sample size, and lack of long-term data beyond four years post-transplant. Prolonged ischemia times and higher transfusion volumes have previously been reported to be associated with poorer outcomes and allograft dysfunction. Whether this could affect longer term (10 year) graft survival remains unclear. It is well established in other forms of transplantation, particularly kidney transplants, that exposure to multiple blood donations results in alloimmunisation to human leucocyte antigens resulting in higher rates of acute rejection and poorer long-term graft survival (100). As an immunoregulatory organ, liver grafts have higher immunotolerance compared to other organs such as the kidney, heart, and intestine. Despite this however exposure to alloantibodies while better tolerated remains a negative prognostic factor. A 2007 study demonstrated that a history of alloantibodies against red blood cell antigens was associated with overall poorer survival (64% vs 87% at 3 years) (101).

While improvement in surgical technique and haemostatic instrumentation can mitigate these complications to a degree they cannot be fully avoided. There is however ongoing innovation in graft preservation following harvesting and prior to transplantation. Previous innovations with the introduction of University of Wisconsin (UW) cold storage solution in Australia in the mid-1990s when it replaced Euro Collins solution significantly improved organ preservation and long-term outcomes. With the view that a good-quality graft is essential for the best outcome, contemporary studies are exploring outcomes of normothermic machine perfusion of liver grafts in repeat liver transplantation compared to contemporaneous cold storage. Initial studies have been performed with the view of increasing availability of organs for adult patients who often spent longer on waiting lists awaiting a graft of appropriate quality with the working theory that more steatotic grafts when preserved with normothermic machine perfusion may reach an appropriate quality threshold with better preservation during transit. Results so far are promising in small groups demonstrating no difference in 6-month graft survival rates despite the normothermic machine perfusion grafts being

significantly more steatotic (102). While larger studies are clearly required this result is promising for reducing the risk associated with longer ischaemic times in this group.

The surgical challenges of repeated liver transplantation may cumulatively add to the risk of a cohort already at risk for long term chronic renal disease. Increased warm ischemia time has been associated with increased risk post-transplant which is concerning, however, the extent of risk in this group is still unknown. It is pertinent to be vigilant with monitoring, utilise protocols such as renal sparing induction regimes in the immediate post-transplant period and in the immediate and long term strictly control blood pressure and calcineurin inhibitor levels. Some studies have suggested early Mycophenolate Mofetil and mTor-I inhibitors may be helpful as adjuncts to keep calcineurin inhibitor levels low with the trade-off of an increased risk of rejection episodes (103,104). A lack of experience in children and uncertainty regarding the long-term risk makes adopting these strategies challenging. While patients are on glucocorticoids strict control of blood sugar levels is pertinent and renal dosing and avoidance of nephrotoxic drugs needs to be kept in mind. Some studies have suggested dietary interventions (salt and protein restriction) may be advisable however this is particularly challenging in the paediatric group and cannot be at the expense of nutritional optimisation and growth targets. Children who do develop end-stage renal disease with a need for dialysis benefit from renal transplantation (105).

While data was limited, this study suggested that neurodevelopment and psychological issues were near universal in this cohort. This is not surprising given the combination of chronic illness, medication effects and complex, long surgical procedures. An isolated consideration of multiple transplantations in the context of patient and graft survival is reassuring however consideration of the morbidity is sobering. This is a particularly challenging area of study given small sample sizes and the challenges of obtaining formal psychometric testing before and after transplantations. Data in this area continues to be scarce, however; since publication one study citing this paper has explored

factors contributing to neurodevelopmental outcomes post liver transplantation. Intraoperative cerebral tissue oxygen saturation and pre-operative weight were found to be statistically significant contributants (50). The former may be modifiable using cerebral tissue oxygen saturation to guide volume resuscitation in conjunction with traditional volume monitoring to ensure adequate cerebral perfusion. The latter is further explored in this thesis's future directions (Page 174). Awareness for the transplantation team and family counselling of the risks are essential. This remains an area of significant need with further research and innovation required.

This study adds to the literature and current debate regarding repeated liver transplantation in children. In the context of lack of other definitive management options and comparable short to medium term survival metrics liver retransplantation is an option to offer families in the context of a discussion highlighting limited outcome data. Given the ongoing improvements in outcomes and survival in children post-transplant this cohort will continue to expand. Studies concentrating on 10-year outcomes, morbidity, and innovations in technique to minimise the associated risks require future study.

3.3.3 Islet Cell Transplant for Pancreatic Disease

See Appendix 1.2 page 198.

CHAPTER 2 – INCLUDED PUBLICATIONS

1. Novel Aetiologies

1.1. Kelgeri C, Couper M, Gupte GL, Brant A, Patel M, Johansen L, Valamparampil J, Ong E, Hartog H, Perera MTPR, Mirza D, van Mourik I, Sharif K, Hartley J. Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med. 2022 Aug 18;387(7):611-619. doi: 10.1056/NEJMoa2206704. Epub 2022 Jul 13. PMID: 35830627.

Clinical Spectrum of Children with Acute Hepatitis of Unknown Aetiology

Chayarani Kelgeri, M.D., M.R.C.P.C.H.[1], Michael Couper, M.B.Ch.B., F.R.A.C.P.[1], Girish L. Gupte, M.D. F.R.C.P.C.H.[1], Alexandra Brant, M.Sc.[1], Mitul Patel, M.D., F.R.C.Path.[2], Lauren Johansen, M.B.B.S., M.R.C.P.C.H.[1], Joseph Valamparampil, M.D., M.R.C.P.C.H.[1], Evelyn Ong, F.R.C.S., P.G.C., H.C.L.[1], Hermien Hartog, Ph.D.[1,3], M.T.P.R. Pereira, M.D., F.R.C.S.[1,3], Darius Mirza, M.S., F.R.C.S.[1,3,4]; Indra Van Mourik, M.D., M.R.C.P., F.R.C.P.C.H.[1], Khalid Sharif, F.R.C.S., F.C.P.S.[1], Jane Hartley, M.R.C.P.C.H., M.Med.Sc., Ph.D.[1]

[1]Liver Unit (including small bowel transplantation), Birmingham Women's and Children's Hospital, United Kingdom

[2]Department of Microbiology, Birmingham Women's and Children's Hospital, United Kingdom

[3]Liver Unit, Queen Elizabeth Hospital, United Kingdom

[4]Centre for Liver Research, University of Birmingham, United Kingdom

Corresponding Author:

Chayarani Kelgeri M.D., M.R.C.P.C.H.

Liver Unit (including Small Bowel Transplant)

Birmingham Women's and Children's NHS Foundation Trust

Birmingham B4 6NH

United Kingdom of Great Britain and Northern Ireland

Abstract

Background:

Beginning in January 2022, there has been an increase in reports of acute hepatitis of unknown aetiology in children across multiple continents, with majority being reported in the United Kingdom (UK). Investigations are ongoing to identify the causative agent(s).

Methods:

This retrospective study includes children referred to a single paediatric liver transplant centre in the UK between 1st January 2022 and 11th April 2022. Cases met the UK Health Security Agency's confirmed case definition of acute hepatitis not due to Hepatitis A-E, metabolic, genetic, congenital, or mechanical cause with serum transaminase > 500 IU/L and age ≤10 years. We reviewed medical records and documented demographics, clinical features, liver biochemistry, serological/ molecular tests for hepatotropic and other viruses, radiology, and clinical outcomes. The outcomes were classified as improving, liver transplantation, or death.

Results: 44 children satisfied the confirmed case definition, and all were previously healthy. Median age was 4 years (range, 1-7years). Common presenting features were jaundice (93%), vomiting (54 %) and diarrhea (32%). 27 of the 30 patients (90%) who had molecular testing for adenovirus were positive. 6 (14%) patients developed fulminant liver failure and received a liver transplant. No patient died. All children, including the 6 who received liver transplants, have been discharged home.

Conclusions:

In this series of 44 children with acute hepatitis of uncertain aetiology, children affected were young with a median age of 4 years, and 90% of those tested were positive for adenovirus; 86% recovered with native liver and 14% required liver transplantation.

Introduction

The World Health Organisation (WHO) first published a disease outbreak notification on the 15th of April 2022, after 10 cases of severe acute hepatitis of unknown aetiology in children were reported across central Scotland between January 2022 to March 2022 [1]. WHO further reported at least 650 probable cases across 33 countries, from the Eastern Mediterranean, European, Pan-America, South-East Asian and Western Pacific region diagnosed between 5th April 2022 and 26th May 2022[2]. Majority of the cases (n=222, 34%) are from the United Kingdom (UK).

UK Health Security Agency (UKHSA) technical briefing released on 19th May 2022 provides an epidemiological update reporting 197 confirmed and possible cases reported between 1st January 2022 to 16th May 2022 in the UK[3]. Cases presented with jaundice (68.8%), vomiting (57.6%), pale stools (42.7%) and gastrointestinal symptoms- diarrhoea (43.1%), nausea (25.7%) and abdominal pain (36.1%). All children were negative for common infective causes: Hepatitis A-E, Cytomegalovirus (CMV), Epstein Barr Virus (EBV), with no common toxin exposure or travel history. 11 children (6%) required Liver transplant (LT)[3]. Public health agencies across the world are collaborating, and investigations are ongoing to delineate the aetiopathogenesis of this illness. Of the 197 UK cases reported in the UKHSA technical briefing report, 179 had molecular testing for adenovirus ,116 (65%) were positive and a possible relationship is being investigated[3].

We work at one of the three specialised paediatric liver transplant centres in the UK, providing ongoing medical advice to manage children with liver disease in regional hospitals via the National On-Call Referral System (NORSe). Children in acute liver failure or likely to progress to acute liver failure are transferred to our centre for further inpatient care. In this

report, we describe the clinical presentation, course of illness and early outcomes of children with acute hepatitis of unknown aetiology who were referred to our centre from different regions of UK. The UKHSA was informed of all cases, and this cohort was included in the pooled analysis reported in their technical briefing and case update release[3].

Methods

All Children ≤16 years of age referred to our unit between 1st January 2022 to 11th April 2022 were screened and those ≤ 10 years of age with acute severe hepatitis as per the confirmed case definition of UKHSA (a person presenting since 1 January 2022 with an acute hepatitis which is not due to hepatitis A-E viruses, or an expected presentation of metabolic, inherited, or genetic, congenital, or mechanical cause with serum transaminase greater than 500 IU/L who is 10 years old and under) were included in this report[3]. Demographic, biochemical, and radiological data were collected from NORSe records and inpatient notes. Full blood count, liver biochemistry, coagulation screen, and tests for aetiological diagnosis- Viral serology for Hepatitis A-E, molecular testing for Cytomegalovirus, Epstein Barr Virus, SARS-CoV-2, Parvo virus and Adenovirus infection in blood, faeces and respiratory (when available) were recorded. The interval from onset of jaundice to peak serum bilirubin and Alanine Transaminase (ALT) was recorded. Some patients had additional investigations for raised liver transaminases which included ferritin, serum alpha 1 antitrypsin level, coeliac screen, serum Immunoglobulins, autoantibodies, ceruloplasmin, serum paracetamol levels, and these were recorded when available. All children underwent abdominal Ultrasound, and liver histology was included if biopsied or explanted for liver transplant. Histology was analysed following application of Haematoxylin Van Gieson, reticulin stain, orcein, Periodic acid-Schiff, Periodic acid-Schiff-diastase, Perls Prussian blue and viral immunostains.

Acute Liver failure was defined as coagulopathy (not corrected with Vitamin K) of PT > 15 seconds/ INR > 1.5 with encephalopathy or PT >20 seconds and INR > 2 with or without encephalopathy[4].Patients were managed as per our Acute Liver failure (ALF) protocol which included intravenous broad-spectrum antibiotics, antifungals, proton pump inhibitor, Vitamin K, restriction of fluids to 70 % maintenance with dextrose to maintain normoglycemia. Children not in ALF received supportive care with oral fat-soluble vitamins (Vitamin K, A, D, E), Ursodeoxycholic acid and were monitored closely with blood tests to identify deterioration. Children with Acute Liver Failure were listed under National Health Service Blood and Transplant (NHS BT) 195/7 UK policy, super urgent category 6 for a liver transplant[5]. This category prioritises acute liver failure over other indications of liver transplant. Cidofovir was used only in post-transplant children if whole blood adenovirus PCR was > 500 copies/ml.

Clinical outcome was recorded in 3 categories i) Improving: Resolving liver dysfunction (consistent fall in bilirubin and transaminases with normal coagulation), ii) Liver transplant iii) Death. The number of days from initial presentation to listing for liver transplant, time from jaundice to encephalopathy, date of transplant, post-transplant adenovirus viraemia and antiviral treatment with Cidofovir were recorded in the transplant group. UKHSA was informed of all cases, and this cohort was included in the pooled analysis reported in their technical briefing[3].

Results

Of the 50 children with acute hepatitis referred to our centre between 1st Jan 2022 to 11th April 2022, 44 satisfied the case definition of a confirmed case (Figure 1). Of the 6 children excluded, 3 were positive for Hepatitis A, one EBV, one Streptococcal sepsis and one had

transaminases < 500 IU/L. 13 of the 44 children required transfer to our centre, and the rest were managed locally. The number requiring admission to our centre and the number eventually requiring transplant from January to April in 2022 was substantially higher than the numbers in the prior years between 2012 and 2021. From January to April in 2022 there were 13 patients admitted to our centre with acute hepatitis of unknown aetiology, compared with 1 to 5 during the same months in prior years. (Figure 2). None of the 44 had any chronic health conditions except for three children. Of these 3 children, one had Cow's milk allergy, one had constipation, and one was autistic. Cases had wide geographic distribution across the UK (Figure S1), and where records were available (80%), all were Caucasian. Children came to medical attention predominantly because of jaundice (93%) (Table 1). Other common presenting features included vomiting (54%), diarrhoea (32%), abdominal pain (27%), lethargy (23%) and these were present at median of 3 days (range,0-42 days) before onset of jaundice.

Clinical examination findings were documented in 34 children and included icterus in 24, hepatomegaly in 18, normal abdominal examination in 10 and a completely normal clinical examination in 2.

The demographics and Liver biochemistry recorded at presentation and peak values are shown in Table 2.

For the 38 children who did not require a liver transplant, the interval from identification of jaundice to peak bilirubin was a median of 5 days (range1-11 days) and to peak ALT, 3 days (range 1-8 days). 16 children who developed a PT> 15 seconds did after a median of 2.5 days (range 1-9 days).

The interval from jaundice to peak bilirubin, ALT and PT were not analysed in the transplanted group as the natural history of the disease is altered by transplantation. All patients with PT ≤20 seconds recovered spontaneously while 6 patients, who progressed to PT > 20 secs despite administration of Vitamin K eventually needed a transplant. The interval from Jaundice to PT >20 seconds was recorded at median of 7 days (range 5-9 days) in the transplanted group.

The full blood count was available in 31 patients and was unremarkable in 30 patients. One patient had low haemoglobin and low neutrophils at presentation. They required a single blood transfusion and 3 days later recovered to normal counts. Renal function remained normal in all patients.

Ferritin was available in 12 children and ranged 31-5082ug/L (normal range 5.3-99.9 ug/L).

Serum Immunoglobulins were available in 34 children, of which 32 were in the normal range (5.46-16.05 g/L) and 2 higher than normal at 18 and 20 g/L. These 2 children were in the non-transplant group. They were retested at follow up and had normal immunoglobulin levels. The autoimmune profile was available in 35 children; 28 had normal results, 7 had positive Anti-nuclear antibody (1:80 titre in one patient and the rest reported as weakly positive), and 6 had positive Smooth muscle antibody (one titre reported as 1:40 and the rest as weakly positive). Serum ceruloplasmin was tested in 12 children, Paracetamol levels in 7, Alpha 1 antitrypsin levels in 5, Coeliac screen in 8 and were all documented in the normal range.

We do not have data on previous infection with SARS-CoV-2 infection in our cohort except for one who had a positive test 6-8 weeks prior to presenting with hepatitis. None of the children to our knowledge, had received the SARS-CoV-2 vaccine. Of the 39 children who

had molecular testing for SARS-CoV-2 during their evaluation, 11 (28%) tested positive. Of the 13 children who had SARS-CoV-2 antibody testing, 5 (38%) tested positive.

Of the 30 patients tested for adenovirus, 27 (90%) were positive (Table 3). Sampling for Adenovirus was from one or more sites- blood, stools, and respiratory secretions. Adenovirus detection was higher in blood (93%) than in respiratory samples (71%) or stools (37%). Two patients who tested positive for adenovirus in stool and respiratory secretions (1 patient each) were both found to be negative on blood, but a false negative cannot be ruled out as the type of sample being tested (blood or serum) was not known. The median Adenovirus PCR was 2733 copies/ml (ranging from detectable but below level of quantification to 39445) in the non-transplanted group(n=22) as compared to median of 20,772 copies/ml (range 3798-29594) in the transplanted group(n=5). Other viruses were tested infrequently (Table 2). All children were negative for CMV. EBV viral capsid antigen and nuclear antigen were positive in 2 and 1 cases respectively.

Abdominal ultrasounds were performed on all 44 children, and the findings are reported in Table 1.

Most patients (n=38, 86.3%) in our cohort demonstrated spontaneous improvement, while some (n=6, 13.6%) continued to worsen, progressing to ALF, and required liver transplant.

In the transplanted cohort(n=6), 5 children had rapidly progressive encephalopathy, with jaundice to encephalopathy interval of 6-7 days. The median interval from listing to transplant was 2 days (range 0-5 days). All transplanted patients received a cadaveric reduced or split left lateral segment liver graft, and the donor criteria was stretched to facilitate the acuity of listed children.

Four of the 5 children with post-transplant adenovirus viremia were treated with Cidofovir until the adenovirus PCR was < 500 copies/ml. The viral load dropped to < 500 at median of 8 days (range 2 – 16 days) after transplant. No patients in this cohort received steroids or any other immunomodulating agents and no changes were made to the usual immunosuppressive regimen (Interleukin-2 receptor antagonist at induction, long term Tacrolimus and Azathioprine). One child, transplanted early in the year did not receive Cidofovir and had adenovirus viraemia up to 26 days after transplant when tested retrospectively on stored blood samples.

Liver histology was available in 9 children (6 explanted livers and 3 liver biopsies). Explanted livers were small, smooth, and grey with bile staining of the cut surface. Microscopy demonstrated distorted architecture with portal tracts containing appropriately sized bile ducts with mild to diffuse inflammation consisting of lymphocytes, plasma cells and eosinophils. Lymphocytes were predominantly CD8 positive T-cells with few CD4 T cells and CD20 positive B cells. Viable parenchyma showed severe disarray with hepatocyte ballooning, canalicular cholestasis and scattered apoptotic bodies. Histology was similar in all biopsies, however, pan acinar necrosis was focal in children who recovered while sub massive necrosis with parenchyma replaced with sheets of macrophages was seen in explants. None had viral inclusions and immunohistochemistry for adenovirus was negative. One biopsy specimen was checked for Epstein-Barr encoding region (EBER) in situ hybridization and showed very occasional positive lymphocyte in sinusoids (1 per high power field). There was no fibrous tissue or evidence of underlying chronic liver disease (Figure S2). Iron, copper associated protein and PAS-D positive globules were not seen. Adenovirus PCR assay targeting the hexogene has been reported in 3 of the 6 explant homogenates to date and are positive. Nucleotide sequencing of the PCR product indicates

the sequence has close similarity to adenovirus serotype 41F. Liver is a vascular organ and further analysis is required to elucidate if this positivity is because of blood. Metagenomics undertaken by UKHSA on blood and liver tissue has detected adeno-associated virus 2(AAV-2) and Herpes virus amongst others and their significance is being further explored[3].

Discussion

We report case series of 44 children with acute hepatitis of unknown aetiology referred to a UK paediatric hepatology tertiary referral unit 2022. These children were previously well and are relatively young. Though the hepatitis resolved in majority of the patients, the high rates (14%) of progression to hepatic failure requiring transplantation underscore the severity of the illness.

While investigation for a causative agent(s) is ongoing, the clinical picture of prodromal phase, biochemical tests suggesting acute hepatitis and ultrasound findings of gall bladder wall thickening with pericholecystic fluid, abdominal lymph nodes and mild hepatosplenomegaly are consistent with a possible viral aetiology. Extensive viral workup has identified Adenovirus as the most common pathogen, although other viruses too have been identified infrequently. UKHAS reported the adenovirus subtype to be 41F[3]. This subtype is primarily known to cause gastroenteritis and differs in tissue tropism from the subtypes causing respiratory and ocular infections[6,7]. Adenovirus positive tests from routine clinical testing are recorded in a second -generation surveillance system in the UK, and reports of positive adenovirus tests from any sampling site- blood, stool and respiratory - in 1–4-year old children have been reported to be higher from November 2021 to April 2022 compared to previous 5 years[3,8]. However, this comparison needs caution as the testing and reporting procedures are variable and influenced by clinical presentation. Adenovirus

infections are typically self-limiting in immunocompetent children but may cause serious disseminated infection in immunocompromised. A prospective study by British Paediatric Surveillance Unit conducted from 1st January 2014 for 13 months reported Adenovirus as a cause of acute infectious hepatitis in hospitalised children in 6% (5 of 81 cases)[9]. The aetiopathogenesis of the current acute hepatitis in previously healthy children is unclear as the liver histology was negative for viral inclusion bodies and viral immunostains. The working hypothesis includes abnormal host response possibly due to lack of exposure because of the SARS-CoV-2 pandemic lock down, an epidemic with normal adenovirus causing the complications to present more frequently, increased susceptibility to adenovirus because of drug, environmental agent or concomitant co infections with other viruses including SARS-CoV-2[3]. There was no clear history of SARS-CoV-2 infection in our cohort prior to presenting with acute hepatitis but it is difficult to be certain as we do not have documentation of symptoms or testing undertaken. Molecular testing for SARS-CoV-2 was positive in 11 (28%) of the 39 children tested at the time of hospital admission with acute hepatitis. Of the 13 who had serological testing for SARS-CoV-2, 5 (38%) were positive.

The pooled data in the UKHSA technical briefing includes 125 cases from England that were tested for SARS-CoV-2 (polymerase chain reaction or lateral flow). 16 cases were positive,13 on admission and 3 were positive 8 weeks prior to hospital presentation[3]. UKHSA is undertaking retrospective serological testing for SARS-CoV-2 to explore its role in the aetiopathogenesis, but interpretation and correlation of the data may be difficult given the high prevalence of SARS-Cov-2 in the general population[3].

A similar outbreak was reported from October to February 2022 in the United States of America. Nine children in Alabama were identified who had presented with both acute hepatitis and adenovirus viraemia. The pattern observed was similar to our cohort, the median age being 2 years 11 months. All children were reported to be previously healthy and presented with a preceding gastrointestinal illness prior to onset of jaundice. 3 progressed to Liver failure and were treated with Cidofovir and steroids and 2 eventually needed liver transplant[10].

Our cohort did not have very high ferritin levels and other diagnostic features to implicate secondary Hemophagocytic lymph histiocytosis as a contributing factor. The presenting and peak bilirubin, ALT, PT and Adenovirus PCR was higher in the transplanted group as compared to the non-transplanted group, but the small size of the cohort precludes meaningful statistical comparisons.

Antiviral therapy in immunocompetent children for Adenovirus is not supported by randomized controlled trials, however Cidofovir is considered the standard practice in immunocompromised, solid organ and bone marrow recipients[11,12]. While its role in disseminated disease in immunocompetent children is less clear, there are reports of successful use[13]. Children in our cohort had decreasing adenoviral load after transplant with or without Cidofovir (n=4 and n=1, respectively). The interval to achieve a load of < 500 copies /ml was longer in the non-treated case (28 days) as compared to the cases treated with Cidofovir (2-16 days). There is little literature on the recommended duration of treatment. Case reports have suggested ongoing treatment until viral load is undetectable with cessation if side effects are seen[14]. A Consensus clinical framework is now available in the UK to guide professionals looking after these children[15]. It is possible that the

adenovirus may have an immuno pathogenic mechanism of injury and the use of steroids in conjunction with Cidofovir is being explored.

No deaths were recorded. Children who recover without transplant have follow up blood tests in their regional hospitals. Not all follow up results are available to us and to date we have information on 11 patients of which 7 achieved normalisation of liver biochemistry at 4-8 weeks from the time of first hospital presentation. All children have been advised to have ongoing monitoring for aplastic anaemia in line with the Royal College of Paediatrics and Child Health, UK consensus framework clinical guideline[15].

The limitation of our report is that this is a small cohort of patients analysed retrospectively and has some missing data. As more data becomes available, the understanding of the natural history and immunopathogenesis of this illness will help plan interventions. Further studies with metagenomics and immunological investigations of the host are being undertaken by the health authorities in the UK to understand the hepatotropism.

There is currently an increased incidence of severe acute hepatitis in young children and approximately 14% in our cohort have required liver transplant. Although new cases continue to be identified across the UK, there is an overall decline corresponding with the declining trend in adenovirus prevalence among the 1- to 4-year-old age group[8]. Clinicians should be vigilant about children presenting with prodromal illness followed by jaundice. Children should follow diagnostic testing as recommended by public health agencies including molecular testing on whole blood for viruses[16].

References

1. Acute hepatitis of unknown aetiology – the United Kingdom of Great Britain and Northern Ireland. World Health Organisation, April2022. https://www.who.int/emergencies/disease-outbreak-news/item/acute-hepatitis-of-unknown-aetiology---the-united-kingdom-of-great-britain-and-northern-ireland (accessed 02/05/22)
2. Acute hepatitis of unknown aetiology in Children- Multi-Country 27 May 2022 https://www.who.int/emergencies/disease-outbreak-news/item/DON-389 (accessed 30 May 2022)
3. Technical briefing3: investigation into acute hepatitis of unknown aetiology in children in England, 19 May 2022. UKHSA publication gateway number GOV-12265.

   https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1073704/acute-hepatitis-technical-briefing-2.pdf (accessed on 12 May 2022)
4. Squire s RH Jr, Schneider BL, Bucuvalas J et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006;148(5):652-8.
5. POLICY POL195/7. Liver Transplantation: Selection Criteria and Recipient Registration. March 2018 https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/9440/pol195_7-liver-selection-policy.pdf

6. Adenovirus Clinical Overview for Healthcare Professionals. https://www.cdc.gov/adenovirus/hcp/clinical-overview.html (accessed 02 June 2022)
7. K. Rafie, A. Lenman, J. Fuchs, A. Rajan, N. Arnberg, L. A. Carlson, The structure of enteric human adenovirus 41—A leading cause of diarrhea in children. Sci. Adv. 7, eabe0974 (2021).
8. UK Health Security Agency, Research and analysis. Investigation into acute hepatitis of unknown aetiology in children in England: case update.17 June 2022 https://www.gov.uk/government/publications/acute-hepatitis-technical-briefing/investigation-into-acute-hepatitis-of-unknown-aetiology-in-children-in-england-case-update (accessed 18 June 2022)
9. Braccio S, Irwin A, Riordan A, Shingadia D, Kelly DA, Bansal S, Ramsay M, Ladhani SN. Acute infectious hepatitis in hospitalised children: a British Paediatric Surveillance Unit study. Arch Dis Child. 2017 Jul;102(7):624-628. doi: 10.1136/archdischild-2016-311916. Epub 2017 Apr 4. PMID: 28377449.
10. Baker JM, Buchfellner M, Britt W et al. Acute Hepatitis and Adenovirus Infection Among Children – Alabama, October 2021 – February 2022. MMWR Morb Mortal Wkly Rep 2022; 71:638-640. DOI: http://dx.doi.org/10.15585/mmwr.mm7118e1external icon.
11. Dela Cruz CS, Pasnick S, Gross JE et al. Adenovirus Infection and Outbreaks: What You Need to Know. Am J Respir Crit Care Med. 2019 Apr 1;199(7): P13-P14. Doi: 10.1164/rccm.1997P13. PMID: 30932693.

12. Ganapathi L, Arnold A, Jones S et al. Use of cidofovir in pediatric patients with adenovirus infection. F1000Res. 2016 Apr 26; 5:758. Doi: 10.12688/f1000research.8374.2. PMID: 27239277; PMCID: PMC4863673.
13. Dotan M, Zion E, Bilavsky E et al. Adenovirus can be a serious, life-threatening disease, even in previously healthy children. Acta Paediatr. 2022 Mar;111(3):614-619. Doi: 10.1111/apa.16207. Epub 2021 Dec 11. PMID: 34862832.
14. Alcamo AM, Wolf MS, Alessi LJ et al. Successful of Cidofovir in an Immunocompetent Child with Severe Adenoviral Sepsis. Pediatrics. 2020 Jan;145(1): e20191632. Doi: 10.1542/peds.2019-1632. Epub 2019 Dec 11. PMID: 31826930; PMCID: PMC6939840.
15. Early investigation and management of children with acute non -A-E hepatitis, with and without liver failure, RCPCH clinical guidelines.27 May 2022. https://www.rcpch.ac.uk/resources/early-investigation-management-children-acute-non-a-e-hepatitis (accessed on 1 June 2022)
16. Guidance for diagnostic testing of cases with severe acute hepatitis of unknown aetiology in children 25 May 2022.https://www.ecdc.europa.eu/sites/default/files/documents/Guidance_Testing_Hepatitis.pdf (accessed on 1 June 2022)

FIGURE LEGENDS

Figure 1: Total confirmed cases and their outcomes

Figure 2: Hospital admissions for acute hepatitis of indeterminate aetiology and liver transplantation for acute liver failure of indeterminate aetiology, annually from 2012 and 2021, and for the months January-April between 2012 and 2022 (annual data for 2022 not yet available; transplants in 2022 are for January- 11th April)

TABLES

Table 1: Presenting symptoms and ultrasound findings

	n (%)
Presenting Features (n=44)	
Jaundice	41(93)
Vomiting	24(54)
Diarrhoea	14(32)
Pale stools	13(29)
Abdominal pain	12(27)
Lethargy	10(23)
Dark urine	6(14)
Coryza	6(13)
Pyrexia	4(9)
Pruritus	1(2)
Abdominal Ultrasound(n=44)	
Gall Bladder thickening	20(45)
Mild Hepatomegaly	12(27)
Mild splenomegaly	8(18)
Normal	6(14)
Abdominal lymph nodes	6(14)
Abdominal fluid	1(2)
Starry sky appearance*	2(4)

* Starry sky appearance is due to accentuated portal venules with diminished liver parenchymal echogenicity.

Table 2: Demographics and Laboratory results

Data*	**Total cohort (n=44) median(range)**	**Non Transplanted (n=38) median(range)**	**Transplanted (n=6) median(range)**	**Normal range**
Age in years	4(1 - 7)	4(1 - 7)	2(1 - 4)	
Gender				
Female	24	21	3	
Male	20	17	3	
Laboratory tests				
WBC	9(5 - 15)	8(5 - 15)	10(8 - 12)	5-16x109/L
Presenting Serum bilirubin	5.8(0.3-10.7)	5.5(0.3-10.7)	7.6(7-9.4)	0- 1mg/dl
Peak Serum bilirubin	8.3(0.3-17.2)	5.5(0.3-15)	15.9(11.5-17.2)	0-1mg/dl
Presenting ALT	2557.5(53 - 5897)	2475.5(53 - 5086)	2977.5(1798 - 5897)	0-41IU/l
Peak ALT	2858.5(603 - 6279)	2694.5(603 - 5086)	3409.5(2819 - 5897)	0-41IU/l
GGT	122(25 - 348)	125.5(25 - 348)	115.5(59 - 165)	0-25IU/l
Serum Albumin	36(27 - 43)	36(27 - 43)	35(31 - 38)	41-52g/l
Presenting PT	14(12 - 28)	13.25(12 - 19.2)	16.65(15.3 - 28)	9-13 seconds
Peak PT	14.25(13 - 86)	13.75(15 - 19.2)	50(36 - 86)	9-13 seconds

WBC: White cell count, ALT: Alanine transaminase, GGT: Gamma Glutamyl transferase,
PT: Prothrombin time, l: Litre, mg; milligram, dl: decilitre, IU: International units
*Data missing: WBC in 13 patients, GGT and Albumin in 3 patients

Table 3: Pathogens tested

Pathogens (patients tested=n)	Positive(percent)				
		Positive sample site for Adenovirus[1]			
			Blood[2] (n=27)	Faeces (n=3)	Respiratory (n=6)
Adenovirus (n=30)	27(90)	Positive	25(93)	2(67)	4(67)
		Negative	2(7)	1(33)	2(33)
SARS-CoV-2 molecular testing (n=39)	11(28)				
SARS-CoV-2 serology (n=13)	5(38)				
Entero/Rhinovirus(n=9)	5(55)				
Human Herpes Virus 6 (n=10)	2(20)				
Influenza A/B(n=4)	1(25)				
Respiratory Syncytial Virus (n=2)	0				
Human Herpes Virus 7 (n=2)	1(50)				
Parvovirus(n=12)	0				

[1]Only one patient was tested and positive in all 3 sites

[2]Sample type used for testing - if whole blood, plasma or serum is not known

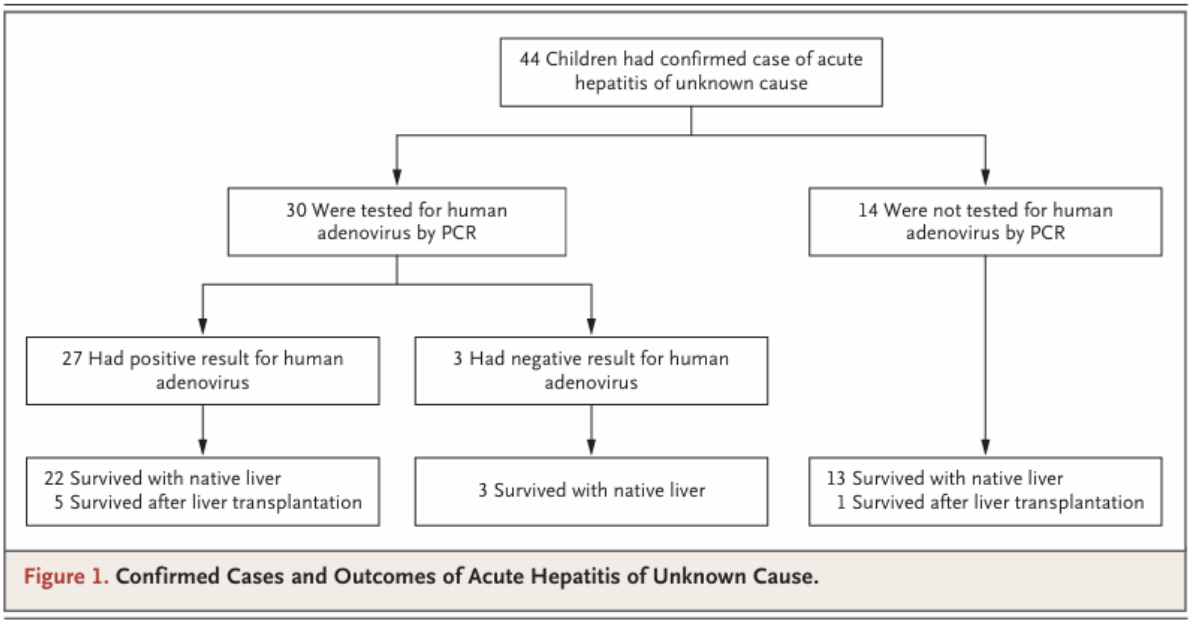


Figure 1. Confirmed Cases and Outcomes of Acute Hepatitis of Unknown Cause.

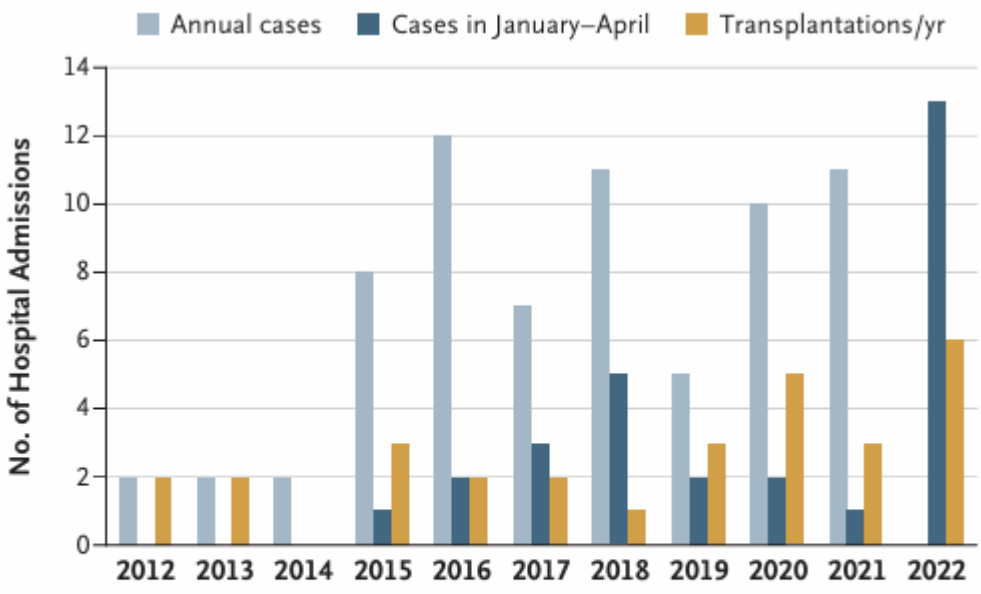


Figure 2. Hospital Admissions for Acute Hepatitis of Unknown Cause and Liver Transplantation for Acute Liver Failure of Unknown Cause.

Annual data from 2012 through 2021 and data for the months from January through April between 2012 and 2022 are shown. Data for transplantations in 2022 are for January 11 through April 11.

1.2. Couper MR, Brown RM, Gupte G, Perera MTPR, Kelgeri C. Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023 Jul 1;77(1):110-114. doi: 10.1097/MPG.0000000000003773. Epub 2023 Mar 14. PMID: 36917836.

Abstract –

GLI-similar 3 (GLIS3) gene mutation heterozygosity is characterised by neonatal diabetes and hypothyroidism. It has wide phenotypic variability. Liver disease is prevalent, and its complications in some phenotypes are life-limiting. Transplantation and the pathogenesis of GLIS3 liver disease are not well explored in the literature. We report two cases of children with GLIS3 mutations with chronic liver disease who required liver transplantation and we present a literature review discussing the pathogenic mechanisms and liver histology. Histology demonstrated predominantly biliary cirrhosis consistent with abnormal bile duct development. Both patients were considered for multi-organ transplantation (liver, pancreas with or without kidney) before receiving a liver transplant alone. Post-operative management can be challenging due to infection, renal disease, and brittle diabetes. GLIS3 mutations need to be added to the list of non-syndromic causes of bile duct paucity in the liver. Liver transplantation should be considered in patients with life-limiting complications related to liver disease.

What is known –

- GLI-similar 3 (GLIS3) mutations are phenotypically broad and liver disease is common.
- Liver disease can be life limiting necessitating consideration of transplantation.
- GLIS3 is a primary cilium-associated protein which is a key site for proteins defective in classical forms of liver and kidney ciliopathies

What is new –

- Histology suggests liver disease in GLIS3 is due to abnormal bile duct development. GLIS3 mutations need to be added to the list of non-syndromic (non-Alagille) causes of bile duct paucity in the liver.

- Liver transplant can be performed successfully however post operative cause may be complicated by renal disease, infection, and challenging diabetic control.

Introduction

GLI-similar 3 (GLIS3) gene mutation heterozygosity is characterised by neonatal diabetes and hypothyroidism. The phenotype is variable. Other associations include liver disease, pancreatic exocrine insufficiency, renal cystic dysplasia, congenital glaucoma, developmental delay, facial dysmorphisms, intrauterine growth restriction, genital abnormalities, skeletal involvement, and rickets. The index cases had early mortality from treatment-resistant endocrine disease and infection1,2.

More recently, milder phenotypes have been recognised with increased life expectancy. Some patients develop chronic liver disease with concomitant portal hypertension. We report two cases of children with GLIS3 mutations with chronic liver disease who required an allogeneic liver transplant and present a literature review to discuss the pathogenic mechanisms and liver histology resulting in their liver disease.

Patient 1

A 5-month-old white male presented with hepatomegaly and conjugated hyperbilirubinemia. He was born to a non-consanguineous couple. His neonatal period was complicated by diabetes, hypothyroidism, pancreatic exocrine insufficiency, conjugated hyperbilirubinemia, and multisystem cystic disease, including both kidneys, mesentery, pancreas, and spleen. PCR analysis of all coding regions and exon/intron boundaries of the GLIS3 gene was successful for exons 5 – 11 but not for exons 1-4, consistent with a homozygous partial gene deletion (c.1? _1710+?del). This result confirmed the diagnosis of GLIS3 neonatal diabetes and congenital hypothyroidism syndrome.

At initial assessment, his blood tests were reported as ALT 221 IU/L (5 – 45), AST 212 IU/L (20 – 60), GGT 859 IU/L (0 – 25), ALP 1782 IU/L (250 – 1000), serum bilirubin 10 umol/L (0 – 21) and serum bile acids 244 umol/L (0 – 14). His management included Ursodeoxycholic acid, Rifampicin for pruritus,

thyroxine, pancreatic enzymes, fat-soluble vitamins, and an insulin pump. Over the subsequent years, his liver disease progressed, and at 6 years of age, he developed portal hypertension with thrombocytopenia and splenomegaly and required one course of oesophageal variceal banding. Surveillance Ultrasound demonstrated abdominal midline cysts. Abdominal MRI and MRCP were performed (see Figure 1). A nuclear medicine TIBIDA scan demonstrated no communication between the cyst and the biliary tree. His mesenteric cysts increased in size, necessitating laparoscopic removal at 6 years of age. These were found to be benign lymphatic cysts on histology.

A liver biopsy at 5 years of age demonstrated severe fibrosis that amounted to established cirrhosis. The picture was distinctively biliary, including patchy paucity of intrahepatic bile ducts but not reminiscent of ductal plate malformation as expected in fibropolycystic syndromes. By 8 years of age, his liver disease had further progressed. His total serum bilirubin and conjugated bilirubin were 110 umol/L and 87 umol/L, respectively. On MRI, there was evidence of renal disease with grossly abnormal kidneys with heterogenous renal parenchyma, cysts, and enlargement; however, renal function tests were normal with Creatinine 30 umol/L (8 – 46), GFR > 100 ml/min/1.73m2 (Urinary Iohexol clearance), urea 4.7 mmol/L (1.7 – 6.7) and Cystatin C 1.14 ml/L (0.56 – 1.30). He had faltering growth (weight < 2nd centile and height 2nd centile for age), portal hypertension, hypersplenism, and metabolic bone disease and was listed for Liver Transplant.

He subsequently underwent a Liver transplant with an ABO-matched split segment II-III graft 3 months following listing. External and cut surfaces of the explant liver were green and showed diffuse fine nodularity. Explant histology varied across the liver. Within both lobes, complete nodules of hepatocytes were surrounded by fibrous tissue indicative of cirrhosis, but only fibrous bridging septa were seen elsewhere. In many portal regions, there was a distinct paucity of bile ducts leaving unaccompanied arteries. These patchy biliary ductules were present focally, sometimes angulated with a complex arrangement vaguely reminiscent of ductal plate malformation. Within the parenchyma was marked canalicular cholestasis, often close to the septa. Normal bile duct and

vessels were seen at the hilum, and the gallbladder wall was oedematous but otherwise normal (Figure 2).

At the time of reporting, he is thriving 14 months post-transplant. Renal function tests remain stable. His immunosuppression was renal sparing- IL2 at induction, with long-term Tacrolimus and Azathioprine. He did not receive glucocorticoids per our standard immunosuppression protocol.

Patient 2

A south Asian female born to a consanguineous couple developed hypothyroidism, neonatal diabetes, polycystic kidney disease, calcium deficiency and rickets in the postnatal period. She was referred to our unit at 1 year of age for cholestatic liver disease. PCR analysis of all coding regions and exon/intron boundaries of the GLIS3 gene was successful for exons 3 – 11 but not exons 1 – 2 (c.-? _388+?del), consistent with a homozygous partial gene deletion. At the initial hepatology assessment, she had predominantly cholestatic disease with biochemical blood tests demonstrating total and conjugated bilirubin of 241 umol/L (0 – 21) and 206 umol/L, respectively, ALP 1360 IU/L (250 – 1000), GGT 177 IU/L (0 – 25), ALT 52 IU/L (5 – 45) and AST 131 IU/L (20 – 60). She was treated with thyroxine, pancreatic enzymes, fat-soluble vitamins, Ursodeoxycholic acid, and cotrimoxazole and had an insulin pump.

Her liver disease continued to progress rapidly, with the development of portal hypertension resulting in life-threatening haematemesis despite variceal banding and sclerotherapy necessitating transplant listing at 17 months of age. At the time of listing, her cholestatic disease had worsened with a total and conjugated bilirubin of 747 umol/L and 614 umol/L, respectively. Liver function tests showed ALP 387 IU/L, GGT 71 IU/L, ALT 65 IU/L and AST 237 umol/L. Her Prothrombin time was 13 seconds, Platelets 140 x 109/L, Albumin 33 g/L and Ammonia 81 umol/L. CT (Computed Tomography) abdomen demonstrated a lobulated cirrhotic liver, portal cavernoma and multiple varices along the

lesser curve of the stomach and gastro-oesophageal junction. The kidneys were enlarged and echogenic with loss of corticomedullary differentiation and multiple small cysts scattered throughout. Renal function was normal with creatinine 14 umol/L (8 – 46), eGFR = 104 ml/min/1.73m2 (Cr EDTA Slope Clearance) and urea 6.8 mmol/L (1.7 – 6.7). She required admission and an ongoing stay in intensive care with respiratory distress and recurrent variceal bleeds while awaiting a graft. She underwent a liver transplant one month following listing with an ABO matched split segment II-III graft. Macroscopically the explant was green and nodular. Histology demonstrated cirrhosis with widespread bile duct paucity. Ductules were seen in modest numbers. The parenchymal nodules were prominently cholestatic. (Figure 3).

Her post-operative period was complicated by the need for prolonged respiratory support for chest infections, diaphragmatic palsy, acute kidney injury and brittle diabetes necessitating a long hospital stay. Initial immunosuppression was with IL2 induction, Tacrolimus and Mycophenolate. She had an episode of biopsy proven acute severe rejection at 15 months post-transplant which was treated with glucocorticoids and basiliximab with subsequent addition of Sirolimus as a long-term maintenance immunosuppressant. Over the next 7 years, graft function remained stable; however, she suffered repeated infections with deteriorating renal function. She subsequently underwent palliative care and died at the age of 9 years.

Discussion

GLIS3 mutations have a variable phenotype, the key features being neonatal diabetes and hypothyroidism. A case series of GLIS3 mutations reported 7 of 12 patients to have liver disease and described the first case to live into adult life. Most patients with liver disease (6 of 7 patients) developed cirrhosis, and liver disease was the predominant cause of mortality outside of the first year of life3. Hence, it is important to consider liver transplant in patients with end-stage liver disease. Simultaneous kidney and pancreas transplant, along with liver transplantation, provide the

best treatment option for all the organ issues involved with the disease; however, these patients present at an early stage in life and organ choices are limited given the constraints of the abdominal domain, unless graft offers are from a similar size matched donor. We describe two patients who successfully underwent isolated Liver transplantation and their explant histology.

The GLIS3 gene codes for a Kruppel-like zinc finger protein which acts as a transcription factor with a critical role as both a repressor and activator and is specifically involved in the development of pancreatic beta cells, the thyroid, eye, liver, and kidney. It is located on chromosome 9p24.2 and is autosomal recessive. It is predominantly expressed in endocrine and preductal progenitors, with lower levels described in the brain, lung, ovaries, and liver3. Before our report, 22 cases from 16 families with 4 allelic variants were recognised in the literature4,5. Our patients had a substantial difference in liver disease progression, with transplantation required at 8 years and 17 months, respectively. Two key mechanisms have been posited for the condition's wide phenotypic variability. Early case reports demonstrated more severe frameshift mutations resulting in a truncated protein compared to less affected individuals with smaller missense or point deletions1. GLIS3 has two dominant transcripts, long (7.5 KB) and small (0.8 – 2 KB), expressed in the pancreas, kidney, thyroid and heart, liver, and skeletal muscle, respectively. Variable involvement of each transcript in each case alters the phenotype6.

There has been no research specifically on the pathogenesis of liver disease in GLIS3, and liver histology is not well described in the literature. Studies of GLIS3 in the pancreas have demonstrated a key role in the maturation of preductal cells into mature ductal cells7. Studies focusing on polycystic kidney disease caused by GLIS3 heterozygosity have demonstrated GLIS3 as a primary cilium-associated protein which is a key site for proteins defective in classical forms of liver and kidney ciliopathies (Congenital hepatic fibrosis, Caroli syndrome and polycystic liver disease). In these conditions, ductal plate malformations are the basis of liver disease. On histology, these conditions classically show abnormal bile duct profiles and progressive fibrosis7,8,9. Our patients had a

predominantly cholestatic picture prior to transplantation. Both our patient's explant histology demonstrated biliary cirrhosis and bile duct paucity, which has not previously been described. In patient 1, there were also areas with prominent biliary structures reminiscent of a ductal plate malformation. In the context of GLIS3 mutations affecting a similar site to other liver/kidney ciliopathies and consistent histology, we propose that the liver disease in GLIS3 is possibly due to abnormal bile duct development.

These patients, at initial presentation, have some similarities to other genetic disorders, such as IPEX, due to FOXP3 mutations. As part of the initial assessment, they should be screened with a neonatal diabetes gene panel and, if negative, progress to whole exome or genome sequencing.

Although anatomically abnormal kidneys, both our patients had normal renal biochemistry and GFR > 80ml/min/1.73m2 at the time of transplant; hence including a renal graft was not necessary. Both of our patients were considered for combined liver and pancreas transplants, given challenging diabetes and exocrine pancreatic deficiency, however this was not thought to be a realistic option. A long waiting time on the transplant list was anticipated because of the lack of availability of size-matched paediatric donors and the technical constraints of a small-sized abdomen. NHS Blood and Transfusion Organ allocation policy in the United Kingdom mandates prioritising small bowel/multi-visceral transplant recipients over other indications and grafts from an adult donor will have technical challenges due to size constraints10. The liver graft will need to be reduced or split whilst keeping the hepatoduodenal ligament with the pancreas and duodenum cap; again, such a large graft would have led to portal vein inflow and alignment issues and other technical complications. Perhaps if the children were older with a bigger abdominal cavity, a composite liver-pancreas graft may have been considered. On the other hand, non composite, liver (orthotopic) and pancreas (heterotrophic) transplant is a technical alternative. In addition, Patient 1 had reasonable diabetic control on an insulin pump and minimal exocrine dysfunction controlled with Creon. It was also anticipated that the diabetic control would improve with the liver glucagon axis working well with a

non-diseased liver graft. The decision for a combined pancreas and kidney transplant will be revisited if he becomes a candidate for a renal transplant. A more complex procedure, transplant course and the need for more substantial immunosuppression would have been inappropriate given the instability of patient 2.

These patients are at particular risk from long-term immunosuppression. Challenging diabetes further increases the risk of infections. Nephrotoxicity from calcineurin inhibitors and poor diabetes control can further exacerbate underlying kidney disease. Both issues were life-limiting in patient 2. A renal-sparing regime utilising Interleukin 2 receptor antagonists at induction is advisable. Particular attention to titrating long-term immunosuppressants to the lowest tolerated dose with diligent monitoring is imperative.

GLIS3 mutations need to be added to the list of non-syndromic (non-Alagille) causes of bile duct paucity in the liver. We can speculate that the mutation affects the embryogenesis of the liver, most likely due to deleterious functioning of the primary cilium. Progressive fibrosis can ensue and become a leading cause of potential morbidity and mortality beyond early life. When considering transplantation, particular care is necessary for these patients, especially in managing immunosuppression, renal disease, diabetes, and exocrine pancreatic deficiency. This report adds to the understanding of liver disease in Glis-3 mutation.

Legends

Figure 1. Sagittal MRI of patient 1, Four cystic structures of variable sizes are demonstrated within the abdomen. The largest measures 81 x 51 mm axially and extends down to the right iliac fossa (arrow). The other three abut the head of the pancreas and the medial wall of the inferior D2/D3 of the duodenum (notched arrow). All three are surrounded by a thin capsule. The liver has a very coarse and heterogenous signal with a nodular and shrunken outline (chevron). Splenomegaly is demonstrated.

Figure 2. Liver histology from patient 1. Top left (HVG x20) shows a less fibrotic area of this liver, there is only one nodule 'N' completely surrounded by fibrous tissue, which elsewhere showed cirrhosis similar to case 2. Top right (H&E x40) shows a relative paucity of portal tracts 'PT' and no fibrosis. Bile duct paucity was evident in most small portal tracts, bottom left H&E x200, all of the structures here are vascular, but there were also areas with prominent biliary structures reminiscent, but not diagnostic of, ductal plate malformation (H&E x100). Figure 3: Liver histology from patient 2. Top left H&E x200 portal tract containing vessels (largest artery 'A' largest vein 'V') but no bile ducts. The absence of a central duct is highlighted with cytokeratin 7 immunohistochemistry (top right x200) there is an artery 'A' a bile duct would be expected in the region of the box but is not seen, a few ductules 'D' are outlined. Very occasional portal tracts did contain a bile duct 'BD' paired with the artery 'A'(bottom left H&E x200). Bottom right panel HVG x20 confirms cirrhosis.

References

1. Dimitri P, Warner JT, Minton JA, Patch AM, Ellard S, Hattersley AT, Barr S, Hawkes D, Wales JK, Gregory JW. Novel GLIS3 mutations demonstrate an extended multisystem phenotype. Eur J Endocrinol. 2011 Mar;164(3):437-43.

2. Alghamdi KA, Alsaedi AB, Aljasser A, Altawil A, Kamal NM. Extended clinical features associated with novel Glis3 mutation: a case report. BMC Endocr Disord. 2017 Mar 2;17(1):14.

3. Dimitri P, Habeb AM, Gurbuz F, Millward A, Wallis S, Moussa K, Akcay T, Taha D, Hogue J, Slavotinek A, Wales JK, Shetty A, Hawkes D, Hattersley AT, Ellard S, De Franco E. Expanding the Clinical Spectrum Associated With GLIS3 Mutations. J Clin Endocrinol Metab. 2015 Oct;100(10):E1362-9.

4. Perdas E, Gadzalska K, Hrytsiuk I, Borowiec M, Fendler W, Młynarski W. Case report: Neonatal diabetes mellitus with congenital hypothyroidism as a result of biallelic heterozygous mutations in GLIS3 gene. Pediatr Diabetes. 2022 Sep;23(6):668-674.

5. Converse P, O'Neill M. OMIM database 610192, GLIS FAMILY ZINC FINGER PROTEIN 3; GLIS3. https://www.omim.org/entry/610192#allelicVariants. Published 06/15/2006, Updated 05/12/2016. Accessed 28/08/22.

6. Wen X, Yang Y. Emerging roles of GLIS3 in neonatal diabetes, type 1 and type 2 diabetes. J Mol Endocrinol. 2017 Feb;58(2):R73-R85.

7. Jetten AM. GLIS1-3 transcription factors: critical roles in the regulation of multiple physiological processes and diseases. Cell Mol Life Sci. 2018 Oct;75(19):3473-3494.

8. Gunay-Aygun M. Liver and kidney disease in ciliopathies. Am J Med Genet C Semin Med Genet. 2009 Nov 15;151C(4):296-306.

9. Chen IY, Whitney-Miller CL, Liao X. Congenital hepatic fibrosis and its mimics: a clinicopathologic study of 19 cases at a single institution. Diagn Pathol. 2021 Aug 30;16(1):81.

10. POL200/5.1 – Introduction to Patient Selection and Organ Allocation Policies. NHS blood and Transplant. https://nhsbtdbe.blob.core.windows.net/umbraco-assets corp/26764/pol200.pdf (accessed 09/10/2022)

Figure 1

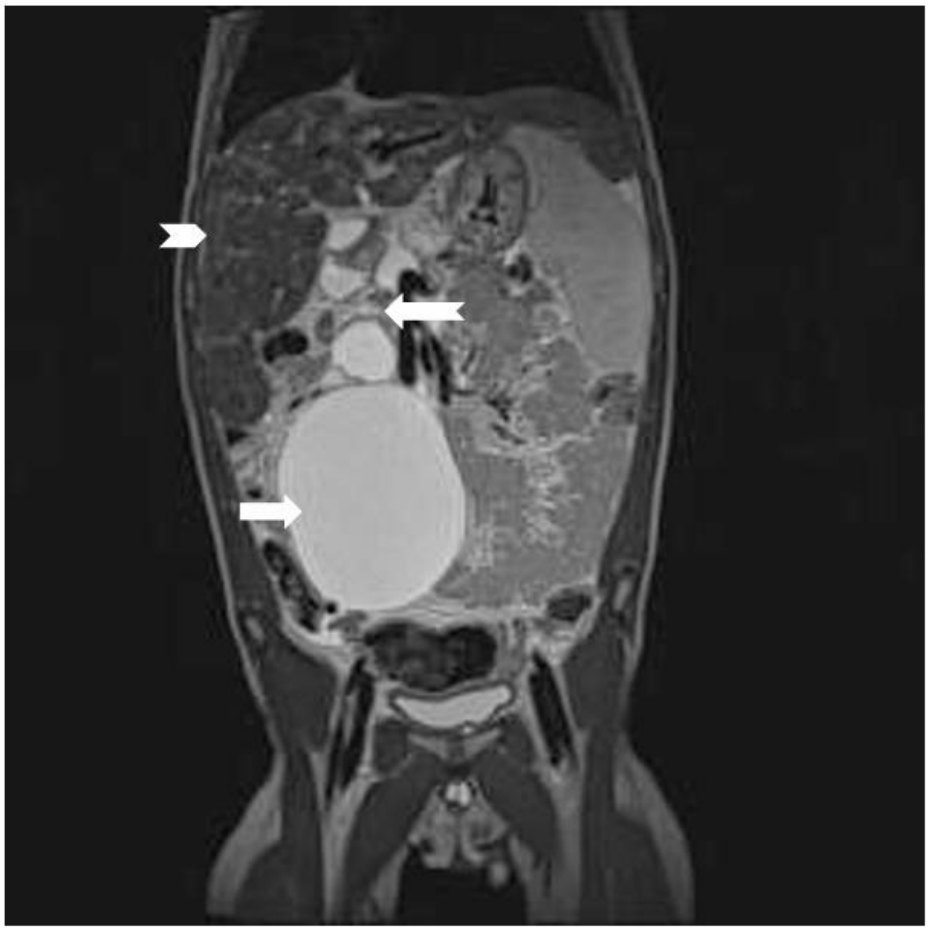


Figure 2

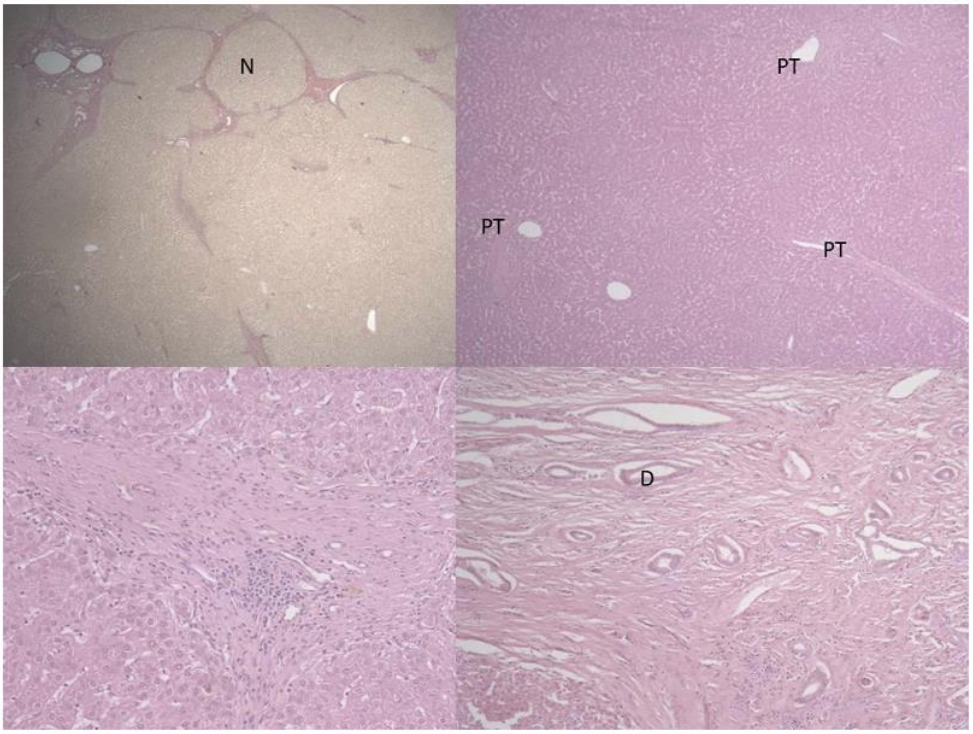
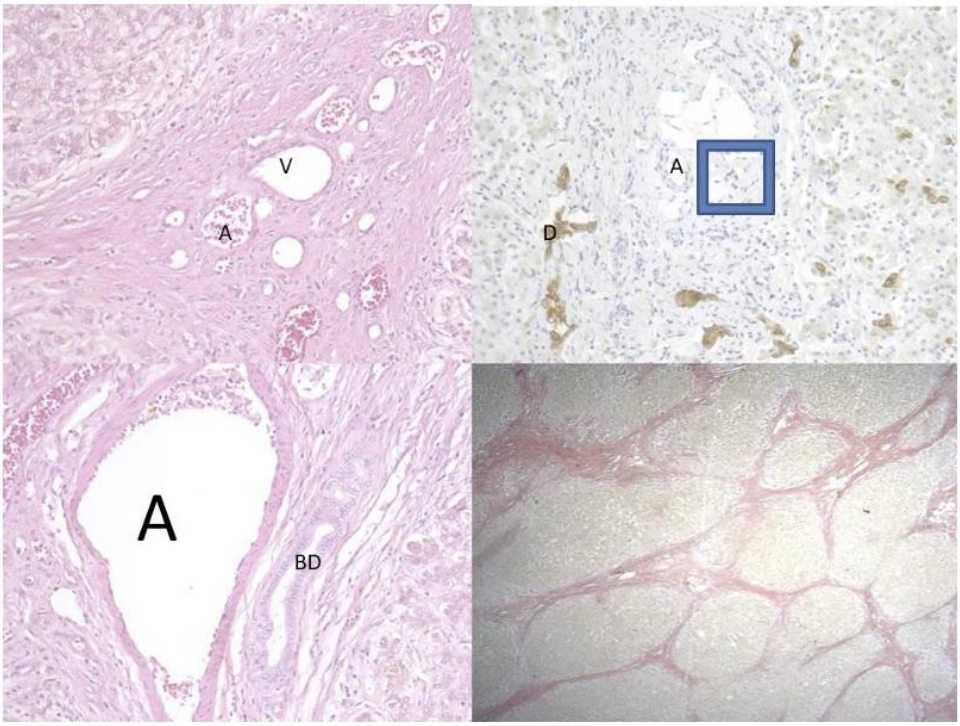


Figure 3



1.3. Couper MR, Eldredge JA, Kirby M, Kirby C, Moore D, Hammond P, Manton N, Glynn A, Couper RT. Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastroenterol Nutr. 2022 Feb 1;74(2):253-257. doi: 10.1097/MPG.0000000000003311. PMID: 34596604.

Abstract

Inflammatory myofibroblastic tumors (IMTs) are rare soft tissue tumors. Reports of gastrointestinal tract, liver and pancreas tumors are limited. The objective of this study is to identify presenting features, contributing prognostic / etiological factors and any variability in outcomes in the context of different historical treatments. We retrospectively reviewed the records of seven children treated at our hospital between 2006 and 2019 and assessed the demographic, presentation, treatment, immunohistochemistry, and outcomes of their tumors. Age range at presentation was 4 months to 15 years with a male predominance. Presentations were typically due to local mass effect or incidental discovery. Systemic symptoms were rare. Outcomes were good with six out of seven stable or in remission irrespective of treatment. Surgical resection where possible is the treatment of choice. Medical therapy had good outcomes with chemotherapy acting as first line treatment when required. The only negative prognostic factor identified was local spread at the time of presentation.

What is known?

- Inflammatory Myofibroblastic Tumors (IMTs) are rare soft tissue tumors that occur mostly in children and young adults.
- Pediatric IMTs are associated with genetic rearrangements particularly 2p and Anaplastic lymphoma kinase (ALK).
- Complete resection is gold standard although adjunctive therapies are documented in case reports.

What is new?

- This is the largest single center series of hepatic, pancreatic and intestinal IMT reported in children.
- Children had good outcomes independent of treatment modality with only one patient developing metastatic recurrence.
- ALK rearrangements may be less common in GI IMTs and their absence did not negatively impact outcomes.

Abbreviations

IMT – Inflammatory myofibroblastic tumor

IQR – Interquartile range

ALK1 - Anaplastic lymphoma kinase

ETV6 - ETS Variant Transcription Factor 6

WBC – White blood count

CRP – C-reactive protein

ESR – Erythrocyte sedimentation rate

CT – Computerized Tomography

FISH – Fluorescence in situ hybridization

Introduction

Inflammatory myofibroblastic tumors (IMTs) are uncommon soft tissue tumors consisting of proliferating myofibroblastic spindle cells and inflammatory infiltrate. They are almost always benign and have a median age of 6 years (IQR 2 to 9.5 years) with an incidence of less than 1 in a million

children1,2,3. The mesentery-bowel and lung-thoracic wall are the most common sites (38.5% of diagnoses each). Hepatic lesions are less common making up only 2% of cases4. Pancreatic lesions are the rarest gastroenterological site with 14 reported pediatric cases 5,6,7. These lesions may result from disordered inflammation with postulated triggers including infections, previous surgery, inflammatory and autoimmune conditions6,8.

Inflammatory myofibroblastic tumors are locally invasive and have a high local recurrence rate (15 – 40%)5,8. Presentations are variable but classically inflammatory symptoms (fever, night sweats and elevated inflammatory markers) and symptoms secondary to local mass effect are observed. The diagnosis is made on histopathology after a mass is identified on imaging, most commonly ultrasound, CT, or MRI or less commonly gastroduodenoscopy in the case of GI IMTs. Tumor histology consists of sheets or entwining bundles of ganglion-like / spindled polygonal cells interspersed with areas of myxoid change with an inflammatory infiltrate of plasma cells, eosinophils, and lymphocytes in a loose stroma with variable degrees of fibrosis and collagen deposition (Figure 1)8,9,10. Management is total resection where possible though successful medical management is described.

This case series describes the experience at the Women's and Children's Hospital, Adelaide with pediatric GI IMTs. We identify presenting features, contributing prognostic / etiological factors and describe outcomes.

Methods

Research ethics committee approval and patient consent for inclusion was obtained. Data was de identified. All patients diagnosed with a gastrointestinal, hepatic, or pancreatic IMT at the Women's and Children's Hospital, Adelaide, Australia from 2006 until October 2019 are reported. Patients were identified from the hospitals pre-existing oncology database. Other than patients with tumors extrinsic to the abdomen no patients were excluded. Medical records, histopathology, radiology, and

operative reports were retrospectively reviewed. All diagnoses were histologically confirmed. Age at diagnosis, gender, presentation, tumor site, past medical history, proinflammatory states (specific infections, previous surgery, inflammatory and autoimmune condition), imaging, histology, Immunohistochemistry (anaplastic lymphoma kinase, ALK1 and ETS Variant Transcription Factor 6, ETV6), FISH results, surgical procedures, medical management, complications, and outcomes were recorded. Minimum follow up was one year.

Results

Presentation ranged from 4 months to 15 years of age with a male predominance (5 male:2 Female). There were four cases of gastrointestinal (stomach, duodenum, small intestine and multifocal abdominal), two cases of liver and one case of head of pancreas IMT (table 1).

The gastrointestinal cases presented with obstructive symptoms, specifically, abdominal pain, distension, vomiting and anorexia. The patient with the head of pancreas IMT presented with poor weight gain and obstructive jaundice. One of the hepatic cases was discovered incidentally in a child undergoing surveillance for Wilms's tumor. The second presented with night sweats, fever, lethargy, and elevated inflammatory markers (WBC, CRP and ESR). Two patients had relevant past medical history (unilateral Wilms' tumor and H.pylori gastritis). No other proinflammatory precedents were noted.

Inflammatory myofibroblastic tumors were identified either by imaging or gastroscopy. Four of seven tumors were identified by ultrasound or gastroscopy or both. Two patients were identified with other imaging studies including an 8-year-old male with a multifocal abdominal lesion who had speckled densities on abdominal X-ray and a 12-year-old female where contrast barium meal and follow through showed hold up in the small intestine. Six of seven patients underwent further cross section

imaging and biopsy to confirm the diagnosis. One patient a12-year-old female with a small intestine IMT was diagnosed after resection.

Immunohistochemistry was performed in all patients with only one case an 8-year-old male with multifocal abdominal IMT demonstrating equivocal staining for ALK-1. This was the only patient with local disease spread and recurrence. One patient a 3-year-old female with Liver IMT demonstrated an ETV6 rearrangement. This patient achieved remission.

Surgical resection was undertaken where feasible. Three cases a 3-year-old female with a liver IMT, a 15-year-old male with a liver IMT and 12-year-old female with a small intestine IMT underwent full surgical resection. All cases that underwent full surgical resection were in remission at the time of reporting. One patient, an 8-year-old male with multifocal abdominal IMT, underwent incomplete resection. Following resection this patient underwent 12 months of chemotherapy (vinblastine and methotrexate) due to local metastasis at diagnosis. They subsequently underwent two further courses of chemotherapy (vinblastine and methotrexate). The first of these courses 12 months after the initial chemotherapy followed further subtotal resection for a new right lower quadrant lesion. The second occurred four years later because of a further right lower quadrant recurrence. Vascular proximity prevented further resection.

Two patients were not amendable to surgical resection due to tumor location and vascular / biliary involvement. The first a 4-month-old male with a head of pancreas IMT underwent a biliary and duodenal bypass followed by cholecystectomy, portoenterostomy and gastrojejunostomy to release biliary obstruction (Figure 1c). This patient subsequently received medical therapy including NSAIDs, dexamethasone and imatinib, a tyrosine kinase inhibitor. A 3-year-old male with a duodenal IMT (Figure 1d) received 12 months of chemotherapy with Vinblastine and Methotrexate. Following discussion regarding treatment options one patient a 10-year-old male with a stomach IMT was managed with expectant surveillance. The 10-year-old male with a stomach IMT and the 3-year-old male with a duodenal IMT have shown tumor regression with two years and ten years follow up

respectively. The boy diagnosed at 4 months with a head of pancreas IMT has a stable tumor fifteen years after diagnosis. All medical therapies were well tolerated. All major complications were due to tumor mass effect, the 4-month-old male with head of pancreas IMT or post-operative complications in an 8-year-old with multifocal abdominal IMT (table 1).

Discussion

Our results demonstrate gastrointestinal IMTs presenting throughout childhood. The head of pancreas lesion in a 4-month male is the youngest afflicted patient. The only other report of an infant with IMT was 6-months-old also with a similar lesion. Both infantile presentation and pancreatic IMTs are very rare11. IMTs have classically been described to present with systemic symptoms (malaise, intermittent fevers, weight loss and night sweats) and elevated inflammatory markers1,5-7,9,12,13. Only one of our patients presented in this fashion. While supportive of the diagnosis these symptoms are non-specific and inconsistent. Our patients most commonly presented with obstructive symptoms. The relevance of IMTs to pediatric gastroenterologists is that because they may present in diverse ways, they need to be considered in the diagnosis of iron deficiency, autoinflammatory states, bowel obstruction and obstructive jaundice. This multiple symptomatic mimicry makes IMTs a rare but important differential diagnosis for many pediatric gastroenterological disorders.

Diagnosis is suggested on imaging and confirmed by biopsy. Imaging usually demonstrates a hypoechoic or hypoattenuating lesion which may be hypervascular with or without calcifications (Figure 1) 1,2,4,6,8,10,12,13. When found endoscopically appearances are typically sessile and polypoid (Figure 1b). Previous studies have suggested 50% of IMTs have rearrangement of the ALK locus on 2p23 and immunohistology has demonstrated ALK staining in 34-56% of cases12,14. The ALK gene encodes a receptor tyrosine kinase with strong oncogenic activity when activated. Coffin et al demonstrated in a cohort of 55 adults and children with IMT in various organ systems that all patients with metastatic disease (6 patients, 1 GI/liver) were ALK negative15. This was not seen in

our cohort. The ETV-NTRK3 fusion oncogene has also been described16. The significance of our single case identified to have ETV6 rearrangement on FISH, in the absence of associated NTRK3 rearrangement is unclear. Our patient did well and achieved remission. Studies have suggested that these lesions may result from disordered inflammation. Postulated triggers include infections, previous surgery, inflammatory and autoimmune conditions however the only relevant contributing past medical history was found in two patients with Wilms' tumor and H.pylori gastritis8,13.

Our experience supports curative resection as a first line treatment. Patients in our cohort treated with full surgical resection had excellent outcomes and remain in remission. When this is achieved our experience and that of others is that no adjunctive therapy is required17. Complete resection results in cure with less than a 10% risk of recurrence5,8. A range of medical therapy has been reported in the treatment of IMTs, however, as they are rare, the efficacy of such treatments is uncertain. Chemotherapy is used in unresectable or relapsed cases. A 2020 oncology study in children with IMTs across all organ systems reported a tumor response in 8 out of 10 patients to combination therapy with Vinblastine and low dose methotrexate17. Our experience has been variable; however, we mostly utilized chemotherapy in our more complex cases. One of our patients a 4-month-old male with a head of pancreas IMT was treated with glucocorticoids, NSAIDs and the tyrosine kinase inhibitor Imatinib after surgery to relieve obstructive symptoms. Imatinib was the only tyrosine kinase inhibitor available on application at that time. This was a historic case and medical therapy was based on reports in the literature at the time5, 18,19. The utility of these management options is questionable. Our patients' lesions remained stable but did not improve. Selective tyrosine kinase inhibitors are therapies of particular interest. The association of oncogenic kinase fusions ALK, ROS1, PDGFRB, ETV-NTRK3 support a model of tyrosine kinase receptor hyperactivity in the molecular basis of IMT20. Phase I/II trials utilizing Critizonib (ALK and ROS1 inhibitor) have showed promise with five of fourteen demonstrating complete response and an incomplete response in a further seven of fourteen patients17.

One of our patients (a 10-year-old male with stomach IMT) underwent surveillance because of a potentially unresectable lesion. Previous reports have advised a period of watchful waiting is reasonable and spontaneous regression has been observed17. In addition, recurrence is less common in the absence of local spread at diagnosis (6% vs 35%)8. However, the risk of spread makes expectant management fraught.

Our study has limitations it is small and retrospective. Pediatric gastrointestinal IMTs are rare and because of this collective experience is important. With all these limitations this is the largest single center study of pediatric GI IMTs.

Conclusion

Pediatric IMT are a rare diagnosis for the Pediatric gastroenterologist. This complicates both management and counselling for these patients. Our cohort suggests whist occasional patients may present with an inflammatory prodrome this is the exception rather than the rule. Diagnosis can reliably be made on histology after lesions are identified on imaging or endoscopy. Historically several treatment regimens have been suggested though confirmation of efficacy is lacking. Despite this variability in our cohort's management, outcomes were generally excellent, with local spread at the time of diagnosis the only identifiable negative prognostic indicator. Currently total resection where possible and chemotherapy are mainstays of treatment. In future selective tyrosine kinase inhibitors and individualized tumor marker characterization and targeting may permit a personalized approach to treatment.

References

1. Sudhan Nagarajan. Somasundaram Jayabose. Inflammatory Myofibroblastic Tumor of the Liver in Children. JPGN 2013;57:277-280

2. Florence Lacaille, Jean-Christophe Fournet. Inflammatory Pseudotumor of the Liver:A Rare Benign Tumor Mimicking a Malignancy. Liver Transplantation and Surgery. 1999;5(1):83-85

3. National cancer institute (NIH) - https://www.cancer.gov/pediatric-adult-rare tumor/rare-tumors/rare-soft-tissue-tumors/inflammatory-myofibroblastic-tumor

4. Rita Alaggio MD, Giovanni Cecchetto MD. Inflammatory myofibroblastic tumors in childhood. Cancer 2010;116:216-26

5. Colangelo M, Di Renzo D, Persico A, Chiesa PL. Case report: Inflammatory myofibroblastic tumor of pancreatic origin in a patient with down syndrome: The role of diagnostic ultrasound. J Ultrasound. 2011;14(1):7-9

6. Liu HK, Lin YC, Yeh ML, Chen YS, Su YT, Tsai CC. Inflammatory myofibroblastic tumors of the pancreas in children: A case report and literature review. Medicine (Baltimore). 2017;96(2):e5870

7. Dagash H, Koh C, Cohen M, Sprigg A, Walker J. Inflammatory myofibroblastic tumor of the pancreas: a case report of 2 pediatric cases--steroids or surgery?. J Pediatr Surg. 2009;44(9):1839-1841

8. Deepak J, Aravind KL, Gowrishankar, Ramesh S. A Case of Rare Small Bowel Tumor in a Child and Review of Literature. Indian J Surg Oncol. 2015;6(3):292-295

9. Omer Saeed, Romil Saxena. Primary mesenchymal liver tumors of childhood. Seminars in Diagnostic Pathology. 2017;34:201-207

10. Cristina R. Antonescu MD. Molecular Characterization of Inflammatory Myofibroblastic Tumors with Frequent ALK and ROS1 Gene Fusion and Rare Novel RET Rearrangements. Am J Surg Pathol 2015;39:957-967

11. Tomazic A, Gvardijancic D, Maucec J, Homan M. Inflammatory myofibroblastic tumor of the pancreatic head - a case report of a 6 months old child and review of the literature. Radiol Oncol. 2015;49(3):265-270. Published 2015 Aug 21. doi:10.2478/raon 2014-0017

12. Stringer MD, Ramani P, Yeung CK, Capps SN, Kiely EM, Spitz L. Abdominal inflammatory myofibroblastic tumors in children. Br J Surg. 1992;79(12):1357-1360

13. Hussong JW, Brown M, Perkins SL, Dehner LP, Coffin CM. Comparison of DNA ploidy, histologic, and immunohistochemical findings with clinical outcome in inflammatory myofibroblastic tumors. Mod. Pathol. 1999; 12: 279–286

14. Walia R, Gjikopulli A, Williams H, et al. Polypoid mass in the ascending colon with intussusception: a rare presentation of an inflammatory myofibroblastic tumor. J Pediatr Gastroenterol Nutr. 2014;58(4):e35

15. Coffin CM, Hornick JL, Fletcher CD. Inflammatory myofibroblastic tumor: comparison of clinicopathologic, histologic, and immunohistochemical features including ALK expression in atypical and aggressive cases. Am J Surg Pathol. 2007;31(4):509-520

16. Yamamoto H, Yoshida A, Taguchi K, et al. ALK, ROS1 and NTRK3 gene rearrangements in inflammatory myofibroblastic tumors. Histopathology. 2016;69(1):72–83

17. Casanova M, Brennan B, Alaggio R, Kelsey A, Orbach D, van Noesel MM, Corradini N, Minard-Colin V, Zanetti I, Bisogno G, Gallego S, Merks JHM, De Salvo GL, Ferrari A. Inflammatory myofibroblastic tumor: The experience of the European pediatric Soft Tissue Sarcoma Study Group (EpSSG). Eur J Cancer. 2020 Mar;127:123-129. doi: 10.1016/j.ejca.2019.12.021. Epub 2020 Jan 30. PMID: 32007712.

18. Przkora R, Bolder U, Schwarz S, et al. Regression of nonresectable inflammatory myofibroblastic tumors after treatment with nonsteroidal anti-inflammatory drugs. Eur J Clin Invest 2004;34(4): 320–321

19. Applebaum H, Kieran MW, Cripe TP, et al. The rationale for nonsteroidal anti inflammatory drug therapy for inflammatory myofibroblastic tumors: a Children's Oncology Group study. J Pediatr Surg. 2005;40(6):999-1003

20. Lovly CM, Gupta A, Lipson D, et al. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. Cancer Discov. 2014;4:889–895.

Figure

Figure 1a - Loosely arranged spindled and stellate cells, admixed with inflammatory cells including plasma cells, lymphocytes with occasional eosinophils. From a pancreatic IMT.

Figure 1b – Gastroscopy macroscopically demonstrating a large sessile polypoid IMT in the duodenum in a 3-year-old male. Also see 1d.

Figure 1c – Axial contrast enhanced CT upper abdomen demonstrating a retroperitoneal soft tissue mass (long arrow) replacing the pancreas and encasing branches of the Coeliac axis (short arrow) in a 4-month-old male.

Figure 1d – Axial contrast enhanced CT upper demonstrating biopsy proven IMT infiltrating the duodenum and extending beyond the serosal surface in a 3-year-old-male.

Table 1: – Demographics, presentation, tumor site, treatment and outcomes of cohort. – *Tyrosine kinase inhibitor

Figure

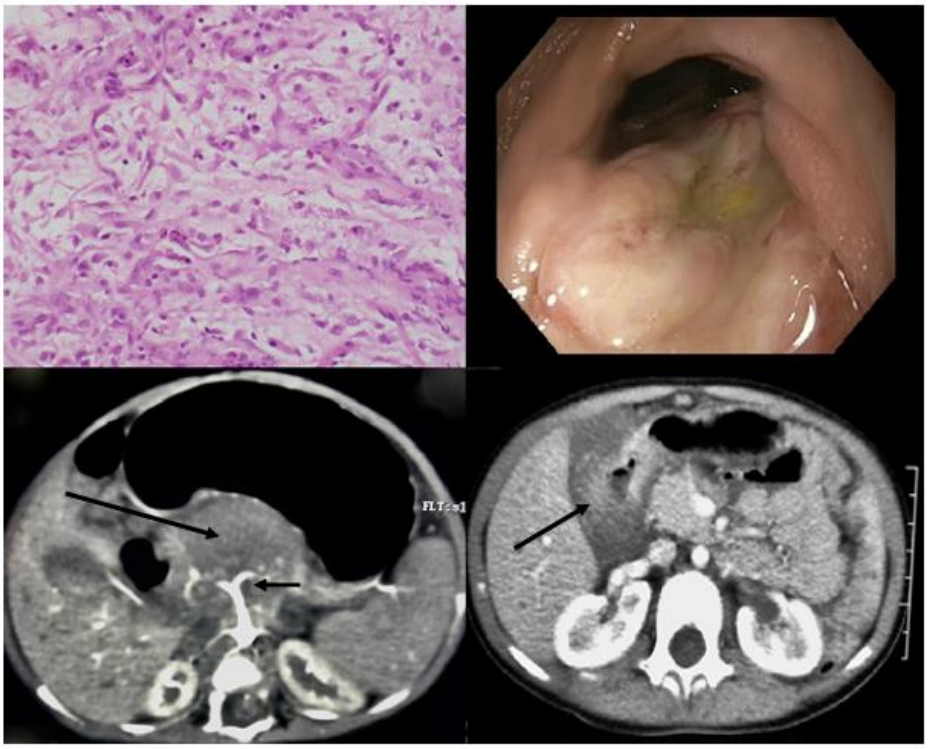


Figure 1a - Loosely arranged spindled and stellate cells, admixed with inflammatory cells including plasma cells, lymphocytes with occasional eosinophils. From a pancreatic IMT.

Figure 1b – Gastroscopy macroscopically demonstrating a large sessile polypoid IMT in the duodenum in a 3-year-old male. Also see 1d.

Figure 1c – Axial contrast enhanced CT upper abdomen demonstrating a retroperitoneal soft tissue mass (long arrow) replacing the pancreas and encasing branches of the Coeliac axis (short arrow) in a 4-month-old male.

Figure 1d – Axial contrast enhanced CT upper demonstrating biopsy proven IMT infiltrating the duodenum and extending beyond the serosal surface in a 3-year-old-male.

Table

Age/Gender	Presentation	Possible pro inflammatory factors	Tumour site	Diagnosis	Immunohistochemistry		Surgical resection	Adjunctive treatment			Complications	Outcome
					ALK-1 expression	ETV6 rearrangement		Chemotherapy	Glucocorticoids	Other		
4 months M	Poor feeding and failure to thrive Cholestatic jaundice	None	Pancreatic head	US, CT and biopsy	No	No	No, location not amenable, see complications	No	Yes	NSAIDs and Imatinib*	Pancreatic duct obstruction Portal vein obstruction and portal hypertension Small bowel obstruction Biliary and duodenal bypass, cholescystectomy, portoenterostomy and gastrojejunostomy	Stable lesion No local or distant spread
3 years M	Vomiting and reduced intake Iron deficiency	Helicobacter Pylori	Duodenum	Gastroscopy, US and CT	No	No	No, location not amenable	Yes, 12 months Vinblastine and Methotrexate	No	No	Haematemesis	Minimal initial change with chemotherapy Slow regression over 9 years follow up
3 years F	Post Wilm's tumor surveillance	Previous Wilms tumour Nephrectomy Chemotherapy (Vincristine and Doxrubicin)	Liver	CT, incidental on tumor surveillance, biopsy	No	Yes	Yes, complete, Segmental liver resection	No	No	No	None	Remission
8 years M	Abdominal pain Vomiting Abdominal distension	None	Jejunum Pelvic mesentery Omentum	X-ray (speckled density), US, CT, Biopsy	Equivocal	No	Yes, incomplete, Jejunal, mesentery and 3 omental deposits resected	Yes, 12 months Vinblastine and Methotrexate	No	No	Bowel obstruction secondary to adhesions Resection of necrotic bowel with subsequent short gut and prolonged PN dependence Anastomotic ulcer bleeding	Two episodes of local recurrence Subtotal resection of right lower quadrant lesion Ongoing chemotherapy for recurrence not amenable to resection
10 years M	Vomiting	None	Stomach	Gastroscopy, US and CT	No	No	No, Opted for surveillance	No	No	No	None	Slow regression with close follow up
12 years F	Epigastric pain Vomiting	None	Small intestine	Contrast meal, US, Histology at resection	No	No	Yes, Complete resection	No	No	No	None	Remission
15 years M	Fever, night sweats and lethargy Arthralgia Raised inflammatory markers	None	Liver	MRI, biopsy	No	No	Yes, Complete resection	No	No	No	None	Remission

Table 1:

- Demographics, presentation, tumor site, treatment and outcomes of cohort.
- *Tyrosine kinase inhibitor

2. Areas of Need and Further Research Within the Specialty

2.1. Couper MR, Brown RM, Nath S, Parida A, Kelgeri C. Periportal necrosis and successful liver transplantation following Lamotrigine drug-induced liver injury in a child. BMJ Case Rep. 2023 Nov 24;16(11):e255787. doi: 10.1136/bcr-2023-255787. PMID: 38000812; PMCID: PMC10679976.

h

BMJ Case Reports

TITLE OF CASE

Periportal necrosis and successful liver transplantation following Lamotrigine drug-induced liver injury in a child

SUMMARY

Lamotrigine is one of the most prescribed anti-epileptics in children and a well-known cause of drug-induced liver injury (DILI). The typical presentation usually includes a drug rash with eosinophilia and systemic symptoms (DRESS syndrome). Cases are typically mild and self-limiting, requiring supportive care only. We report a severe Lamotrigine-induced DILI with a non-typical presentation with hyperammonaemia and rapid clinical deterioration. We present a literature review exploring contributing factors, transplant considerations and liver histology. Histology showed periportal necrosis, which is recognised as a pattern of DILI but has not been previously described with Lamotrigine. Our patient proceeded to transplant and is the first reported liver transplant for Lamotrigine DILI in a child. A directed and rapid diagnostic approach is crucial to avoid delays and rule out multisystemic metabolic and genetic conditions that preclude liver transplantation.

BACKGROUND

Lamotrigine is a rare but well-known cause of idiosyncratic liver injury. The typical presentation usually includes a drug rash with eosinophilia and systemic symptoms (DRESS syndrome)[1,2]. In previous cases involving children, reactions have been more common when Lamotrigine has been given in conjunction with Sodium Valproate[3]. Typically, there is a rapid recovery on cessation[3, 4].

There are limited reports of liver transplantation in Lamotrigine-related liver injury; however, none previously in children[5]. Young children require careful consideration

and an extensive diagnostic workup due to the potential for undiagnosed genetic or metabolic conditions as contributing or unmasked causative aetiology.

We describe a severe idiosyncratic liver injury secondary to Lamotrigine in a child without features consistent with DRESS syndrome and severe hyperammonaemia. We explore the histological findings and specific transplant considerations.

CASE PRESENTATION

A female in middle childhood presented to her local hospital with constipation, new-onset vomiting and increasing lethargy. She had a background of attention deficit hyperactivity disorder and challenging behaviour, including self-harm. Her long-term medications included Atomoxetine, Methylphenidate, Risperidone, and Melatonin, and she had been commenced on Lamotrigine three weeks prior, with the dose being increased 3 days before presentation. There had been no history of rash, fever, facial oedema, or lymphadenopathy. On examination, her GCS was 14 (Opening eyes to speech); she had normal vitals and was quiet and subdued. Initial biochemistry demonstrated an ammonia of 942 umol/L (< 75), ALT 9067 IU/L (5 – 45), total bilirubin 135 umol/L (0 – 21), conjugated bilirubin 110 umol/L (0 – 11), lactate of 10.1 mmol/L (< 1 mmol/L) and glucose of 2.6 mmol/L (> 3.3 mmol/L). The clotting profile showed an INR of 4.9. She was commenced on the acute liver failure protocol with an initial improvement in ammonia to 390 umol/L prior to transfer to the Birmingham Children's Hospital Liver unit. Her regular medications were withheld.

Due to the substantial distance between centres, the transfer took five hours. During this time, she deteriorated. She became increasingly agitated and, on arrival, had a GCS of 8 (E1, V2, M5). Biochemistry on arrival demonstrated ammonia of 204 umol/L, ALT, and AST > 7000 IU/L, GGT 69 IU/L (0 – 25), ALP 286 IU/L (80 – 330), total bilirubin 109 umol/L, conjugated bilirubin 79 umol/L (0 – 11) and, Albumin 36 g/L (43-54). Complete

blood count, including eosinophil count and renal function, were normal. She had a persistent coagulopathy with an INR of 5.2.

She was admitted to the intensive care unit for intubation and to facilitate supportive care. Extensive laboratory workup was sent, including cholestatic, metabolic and mitochondrial genetic panels, whole exome sequencing, toxicology panel, Lamotrigine levels, alpha-fetoprotein, endocrine screen, free fatty acids, cholesterol, triglycerides, alpha-1-antitrypsin, copper, caeruloplasmin, acetylcarnitine profile, carnitine, viral PCR (CMV, Adenovirus, EBV, HSV 1&2, VZV, Enterovirus, Hep A-E), autoimmune panel, immunoglobulins, Gal-1-PUT, urinary organic acids, plasma amino acids and VLCFAs. A high Ferritin of 15832 ug/L (13.7 – 78.8) was found; otherwise, this panel, including genetic studies, was normal. Aside from a diffusely oedematous gallbladder wall and a small volume of ascites, liver ultrasound was normal. An MRI brain, including spectroscopy, demonstrated changes consistent with hepatic encephalopathy and no convincing evidence of a mitochondrial disorder.

During her stay in intensive care, she required increasing support, including haemofiltration. Plasma exchange was commenced for fulminant liver failure due to reports of efficacy in DRESS-Lamotrigine DILI[6]. Due to no improvement, ongoing high supportive care requirements, and no evidence of a systemic metabolic process, she was listed for a super urgent transplant as per the NHSBT (National Health Services for Blood and Transfusion) idiosyncratic drug ALF category[7]. While on the waiting list, she developed hypertension and was found to have a fixed dilated right pupil. A CT of her head demonstrated loss of extra-axial CSF space and small ventricles with a degree of tonsillar herniation consistent with increased intracranial pressure and not previously apparent on MRI brain. She was commenced on 3% hypertonic saline, and her target PCO2 was lowered. Mannitol was administered due to persistent clinical features of raised intracranial pressure despite reaching targeted serum sodium and osmolarity.

She underwent a liver transplant with an ABO-matched reduced graft 6 days after her initial presentation. Macroscopically, the explant was pale and firm. Histology was undertaken with Haematoxylin Van Gieson, reticulin, orcein, periodic acid Schiff (PAS), PAS diastase and perls stains. The explant showed submassive hepatic necrosis with strikingly periportal zonal distribution and bridging portal/portal necrosis (Figure 1). This pattern was highlighted on the reticulin staining in the necrotic regions. The bridging necrosis was also visible on HVG and orcein stains; the staining was only light in necrotic regions against underlying mature fibrosis/chronic liver disease. Surviving hepatocytes showed severe canalicular cholestasis and striking ballooning. Inflammatory cells were seen in the necrotic regions, but in relatively low numbers, lymphocytes were seen with occasional plasma cells and only very rare eosinophils. Haemophagocytosis was not identified. On special staining, there was no evidence of iron deposition, no HBsAg-positive cells were seen, and no DPAS-positive globules (as expected in alpha-1-antitrypsin deficiency) or granules of copper in periportal areas. This pattern was felt to be not entirely specific but most suggestive of a drug-induced liver injury.

Both the transplant procedure and post-operative course were uncomplicated. The immunosuppression regime was with IL2 receptor antagonist, Tacrolimus, and Azathioprine per our unit's standard protocol. At the time of reporting, she has been discharged and is well on follow-up three months following the transplant with no neurological sequelae.

DISCUSSION

1. **Limited data on Paediatric drug induced liver injuries**

   Despite being a common cause of liver injury, the spectrum and prognostic factors in children's drug-induced liver injury (DILI) are poorly established. While

most commonly attributable to antimicrobials and central nervous system (CNS) agents, the wide range of causative agents makes study challenging[8].

Lamotrigine is a widely used anticonvulsant believed to inhibit the release of excitatory neurotransmitters but may also work directly on sodium channels on neuronal cells. It is one of the most prescribed anti-epileptics in children, and liver function monitoring is not routinely performed. Hepatotoxicity is estimated to occur in 1 in 2000 to 10,000 patients. The mechanism is thought to be a hypersensitivity or immunological response to a metabolically generated drug-protein complex. Case reports in the literature describe presentation with DRESS syndrome and commonly eosinophilia, facial oedema, and lymphadenopathy. A hepatic picture is typically seen with mild to moderate increases in transaminases (ALT 128 – 732 IU/L), although more severe cases have been documented[9].

The commencement of Lamotrigine in the context of multiple other CNS-acting agents may have been a contributing factor in this case. Atomoxetine, a long-term medication in our patient, has been reported to cause hepatitis with transaminase elevations often > 20x normal ranges. Atomoxetine is also thought to cause hepatotoxicity due to the production of a toxic intermediate or immunologic by-product. Atomoxetine is extensively metabolised by CYP 2D6, which is strongly inhibited by Lamotrigine[10-11]. Concurrent usage may have been synergistic in causing liver injury, resulting in a rapid and severe presentation.

2. **Challenges of diagnosis in this age group and need to rule out conditions contraindicating transplant**

Acute liver failure in the context of hyperammonaemia, high lactate levels, history of neurological issues and recent commencement of a CNS agent require particular care in children. Conditions such as Alpers-Huttenlocher syndrome classically onset later in childhood and can be precipitated by viral infections and CNS agents, classically Sodium Valproate. A directed and rapid diagnostic approach is crucial to avoid delays and rule out multisystemic metabolic and genetic conditions that preclude liver transplantation.

Histology is often non-specific in determining the cause of acute liver injury. Where the necrosis is zonal, as in this case, DILI is favoured. The effect is usually intrinsic rather than idiosyncratic. For example, a perivenular pattern of zonal necrosis is more commonly seen after paracetamol overdose. Our case showed periportal necrosis, which is recognised as a pattern of drug-induced injury (for example, ferrous sulphate and cocaine DILI) but has not been described with Lamotrigine. This would usually be expected to cause acute hepatitis histologically. Some cases of necrosis have been described, including in a series of sequential biopsies, but not specifically with a periportal pattern[12]. Cholestasis was marked and is a recognised pattern of DILI histologically. Ferritin was elevated; however, in the absence of haemophagocytosis, fever, splenomegaly and normal triglycerides, a diagnosis of Haemophagocytic lymphohistiocytosis was excluded[13]. A high index of suspicion is required in children commenced on a new CNS agent, even in the absence of typically reported features.

3. **Limited transplant experience in this population**

Prognostication and treatment in children with DILI remain challenging. We report a severe case and the first successful treatment of a Lamotrigine DILI with liver transplantation in a child. Despite a severe presentation requiring intensive

supportive care and features of raised intracranial pressure, the outcome was excellent. The transplant procedure was uncomplicated, and as of the time of reporting, there have been no complications or neurological sequelae. Particular care and rapid commencement of treatment and aetiological investigations are crucial, particularly in the context of polypharmacy and with features consistent with metabolic derangement. This report adds to the understanding of Lamotrigine DILI.

LEARNING POINTS/TAKE HOME MESSAGES 3-5 bullet points

What is known –

- Lamotrigine is a commonly prescribed CNS acting agent in children and is associated with DILI.
- Cases are typically mild and self-limiting presenting with DRESS syndrome.
- Histology is typically non-specific or demonstrates a perivenular pattern of zonal necrosis.

What is new -

- Severe presentations and rapid deterioration are possible and a high index of suspicion is required in the context of polypharmacy or new CNS acting agent commencement.
- Histology can demonstrate a periportal necrosis instead of the typical reported pattern.
- Liver transplantation in the absence of multisystemic metabolic and genetic conditions is appropriate.

REFERENCES

1. Makin AJ, Fitt S, Williams R, Duncan JS. Fulminant hepatic failure induced by lamotrigine. BMJ. 1995;311:292.
2. Aouam K, Ben Romdhane F, Loussaief C, Salem R, Toumi A, Belhadjali H, Chaabane A, et al. Hypersensitivity syndrome induced by anticonvulsants: possible cross-reactivity between carbamazepine and lamotrigine. J Clin Pharmacol. 2009;49:1488–91.
3. Chattergoon DS, McGuigan MA, Koren G, Hwang P, Ito S. Multiorgan dysfunction and disseminated intravascular coagulation in children receiving lamotrigine and valproic acid. Neurology. 1997;49:1442–4.
4. Su-Yin AN, Tai WW, Olson KR. Lamotrigine-associated reversible severe hepatitis: a case report. J Med Toxicol. 2008;4:258–60.
5. Amante MF, Filippini AV, Cejas N, Lendoire J, Imventarza O, Parisi C. Dress syndrome and fulminant hepatic failure induced by lamotrigine. Ann Hepatol. 2009;8:75–
6. Alexander, E.C., Deep, A. Therapeutic plasma exchange in children with acute liver failure (ALF): is it time for incorporation into the ALF armamentarium?. Pediatr Nephrol 37, 1775–1788 (2022).
7. National Health Services for Blood and Transfusion criteria, https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/9440/pol195_7-liver-selection-policy.pdf. Accessed 29/01/2023
8. Molleston JP, Fontana RJ, Lopez MJ, Kleiner DE, Gu J, Chalasani N; Drug-Induced Liver Injury Network. Characteristics of idiosyncratic drug-induced liver injury in children: results from the DILIN prospective study. J Pediatr Gastroenterol Nutr. 2011 Aug;53(2):182-9. doi: 10.1097/MPG.0b013e31821d6cfd.

9. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. = Lamotrigine Website - https://www.ncbi.nlm.nih.gov/books/NBK548562/#:~:text=Lamotrigine%20is%20a%20widely%20used,be%20severe%20and%20even%20fatal. Accessed 17-12-2022

10. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. – Atomoxatine, https://www.ncbi.nlm.nih.gov/books/NBK548671/ Accessed 17-12-2022

11. Dodsworth T, Kim DD, Procyshyn RM, Ross CJ, Honer WG, Barr AM. A systematic review of the effects of CYP2D6 phenotypes on risperidone treatment in children and adolescents. Child Adolesc Psychiatry Ment Health. 2018 Jul 16;12:37. doi: 10.1186/s13034-018-0243-2.

12. Overstreet K, Costanza C, Behling C, Hassanin T, Masliah E. Fatal progressive hepatic necrosis associated with lamotrigine treatment: a case report and literature review. Dig Dis Sci. 2002 Sep;47(9):1921-5. doi: 10.1023/a:1019627618972.

13. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007 Feb;48(2):124-31. doi: 10.1002/pbc.21039.

FIGURE/VIDEO CAPTIONS

Upper left H&E x40. Necrosis is seen linking portal tracts ‘PT’, parenchyma around the central vein (‘CV’) is viable. Top right reticulin x40. Collapse of the reticulin framework is seen in the portal/portal necrotic bridges. Bottom left, H&E x200. There is a zone of necrosis, marked by the arrow, around a portal tract. Bottom right H&E x200. Viable

parenchyma around the central vein shows marked canalicular cholestasis and hepatocyte ballooning.

PATIENT'S PERSPECTIVE

- ***Not obtained. Enquiries can be made if felt necessary however this is complicated due to the patient's custody and care arrangements.***

INTELLECTUAL PROPERTY RIGHTS ASSIGNMENT OR LICENCE STATEMENT

Date: 04/04/2023

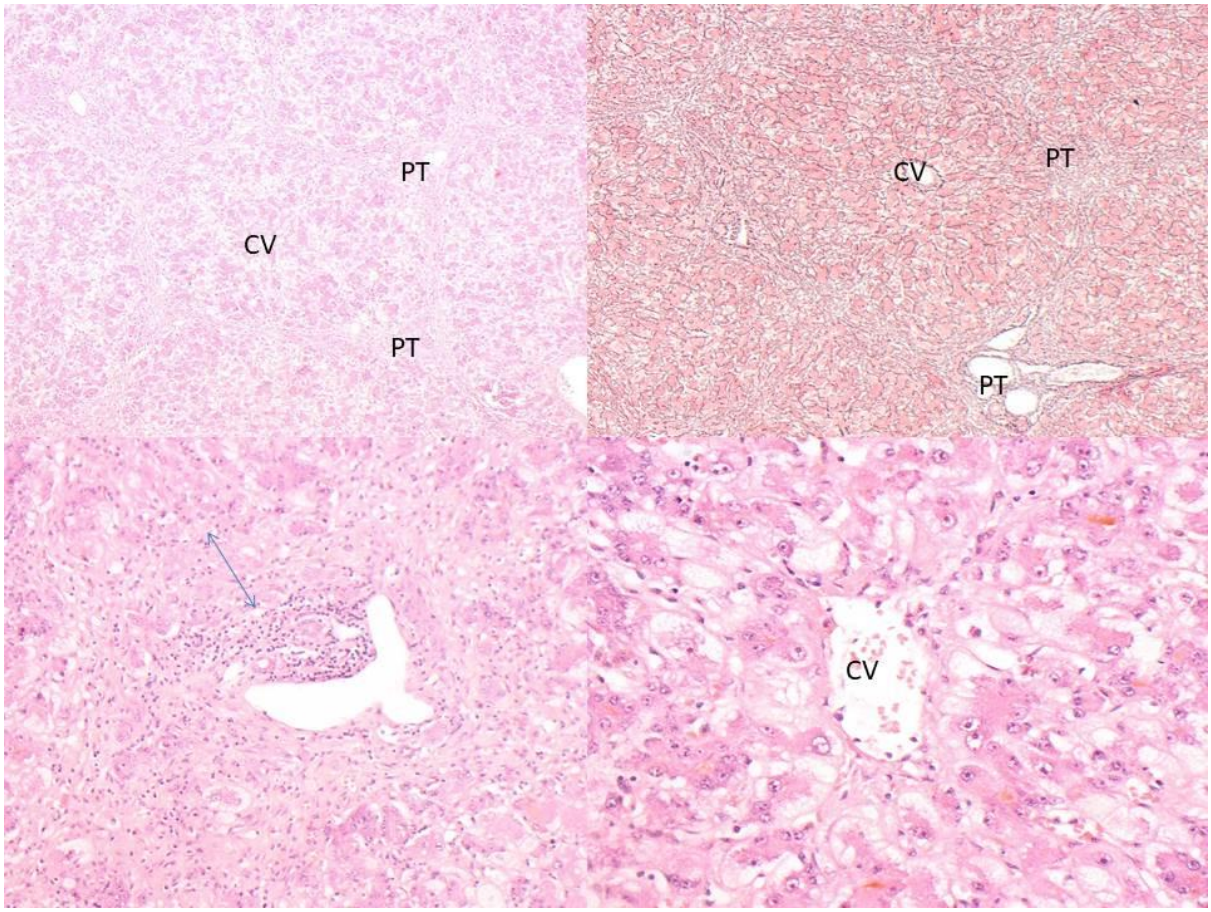


Figure legend; Upper left H&E x40. Necrosis is seen linking portal tracts ‘PT’, parenchyma around the central vein (‘CV’) is viable. Top right reticulin x40. Collapse of the reticulin framework is seen in the portal/portal necrotic bridges. Bottom left, H&E x200. There is a zone of necrosis, marked by the arrow, around a portal tract. Bottom right H&E x200. Viable parenchyma around the central vein shows marked canclicular cholestasis and hepatocyte ballooning.

2.2. Couper MR, Chennapragada M, Magoffin A. Hepatobiliary and Pancreatic: A rare peribiliary lesion. J Gastroenterol Hepatol. 2021 Sep;36(9):2336. doi: 10.1111/jgh.15556. Epub 2021 Jun 29. PMID: 34189779.

Title -

Clinical Challenges and Images in GI – Rare Infiltrating biliary lesion in a child

Keywords –

Biliary stricture, Eosinophilia, Pediatric

Authors –

Michael R Couper, MbCHB

Murthy Chennapragada MBBS FRANZCR

Annabel Magoffin MBBS FRACP

Institutions –

Children's Hospital at Westmead, Gastroenterology

Children's Hospital at Westmead, Radiology

Children's Hospital at Westmead, Pathology

Corresponding Author –

Annabel Magoffin

Annabel.magoffin@health.nsw.gov.au

Conflict of interest –

The authors of this article have no conflicts of interest to declare. This article had no sources of funding.

Question –

A ten-year-old Lebanese boy presented with ten days of intermittent periumbilical pain, lethargy and reduced appetite. He had been pruritic for several months. He had a past history of mild seasonal asthma but was otherwise well. He was Australian by birth but had lived in rural Lebanon for five years prior to the family immigrating back to Australia. On examination there was hepatomegaly with a soft liver edge two centimetres below the costal margin. Laboratory examination revealed deranged liver function tests (Total bilirubin 33 umol/L normal <10, AST 99 U/L normal <50, ALT 227 U/L normal <36, GGT 326 U/L normal <36), raised globulins (37 g/L normal <36) and elevated eosinophils (30.37×10^9/L normal <1.1). Abdominal Ultrasonography (USS) demonstrated multiple dilated intrahepatic ducts up to 6 mm in diameter. Magnetic Resonance Cholangiopancreatography (MRCP) demonstrated dilated intrahepatic ducts within both lobes converging on an ill-defined and T2 hyperintense structure infiltrating along the portal tracts towards the hepatic hilum (Figure 1 - arrowheads). What is the differential diagnosis? How should this be managed?

Answer –

Eosinophilic Cholangitis

Histopathology showed expansion of a majority of portal tracts with fine fibrous tissue, oedema and a mixed infiltrate of lymphocytes, eosinophils and neutrophils (Figure 2). Minimal focal interface hepatitis was present with spill over of inflammation in a minority of tracts, and a variable lobular infiltrate of lymphocytes and eosinophils. At least one large bile duct had concentric fibroblastic changes, mural thickening and oedema. Percutaneous transhepatic cholangiography showed obstructed hepatic ducts (predominantly left lobe) due to stricture from extrinsic compression (Figure 3A). Balloon dilatation of the obstruction followed by internal – external biliary drain insertion was performed (Figure 3B). A trial of glucocorticoids was considered however complete resolution was achieved with expectant management and sustained on drain removal.

Eosinophilic Cholangitis (EC) is a rare benign disease resulting in biliary strictures due to transmural eosinophilic infiltration. It has been suggested Eosinophils produce the cytokine, Transforming Growth Factor-B, inducing fibrosis. Diagnosis can be challenging with differentials including primary sclerosing cholangitis, sarcoidosis, IgG4-related sclerosing cholangitis, mast cell cholangitis, ischemic cholangiopathy, cholangiocarcinoma, parasitic or protozoic infections, follicular cholangitis, choledocholithiasis especially with ascending cholangitis, Mirizzi syndrome and autoimmune pancreatitis type 1 and 2[1,2]. As a malignant masquerade, 66% of reported cases of EC had undergone surgical intervention[2]. Peripheral eosinophilia has been observed in 65% of case reports, however its absence does not preclude the diagnosis[3]. Our patient's eosinophilia rapidly subsided spontaneously prior to drain insertion raising the possibility that eosinophilia may be transient and potentially missed. Krishna Yamuna et al proposed a diagnostic algorithm suggesting the diagnosis can be made with the combination of mass and/or biliary stricture or dilatation on cross sectional imaging and peripheral eosinophilia. In the absence of eosinophilia, ERCP or a percutaneous liver biopsy to obtain tissue for histopathology is recommended[2].

Individual case reports have demonstrated a response to high dose glucocorticoids (Prednisolone or Budesonide) with biliary stenting as needed for obstructive symptoms[2]. Similar to this case, spontaneous resolution has been reported with and without biliary stenting raising the possibility of these patients undergoing expectant management with close observation.

References –

1. Eosinophilic cholangitis: A case report of diagnostically challenging eosinophilic infiltrative biliary obstruction. Dodda A, Matsukuma K, Urayama S. World J Gastrointest Endosc 2019; 11(12): 589-595
2. The Great Imposture: Eosinophilic Cholangitis. Krishna Yamuna T, Schammel Joshua A, Schammel Christine. Surgical Case Reports 2018; 1(3): 2-9
3. Eosinophilic cholangitis is a potentially underdiagnosed etiology in indeterminate biliary stricture. Walter D, Hartmann S, Peveling-Oberhag J. World J Gastroenterol 2017; 23(6): 1044-1050.

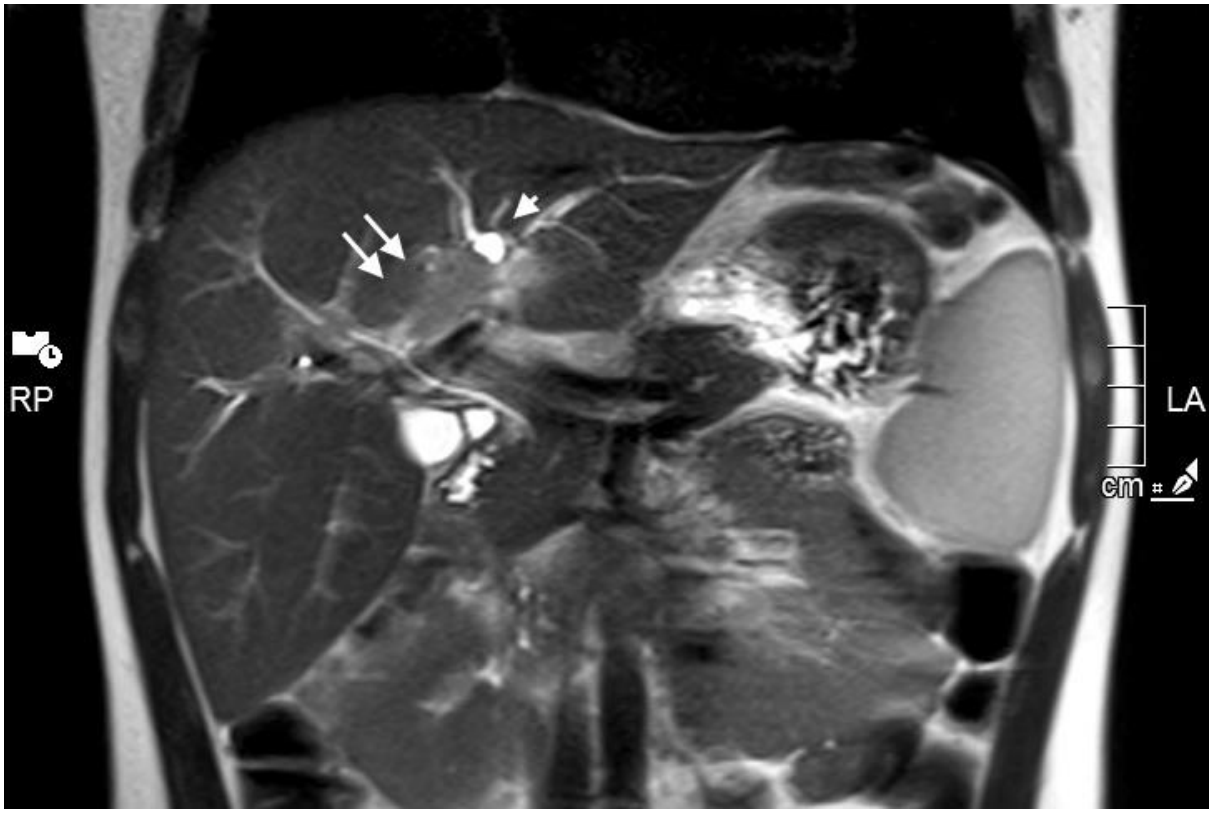


Figure 1

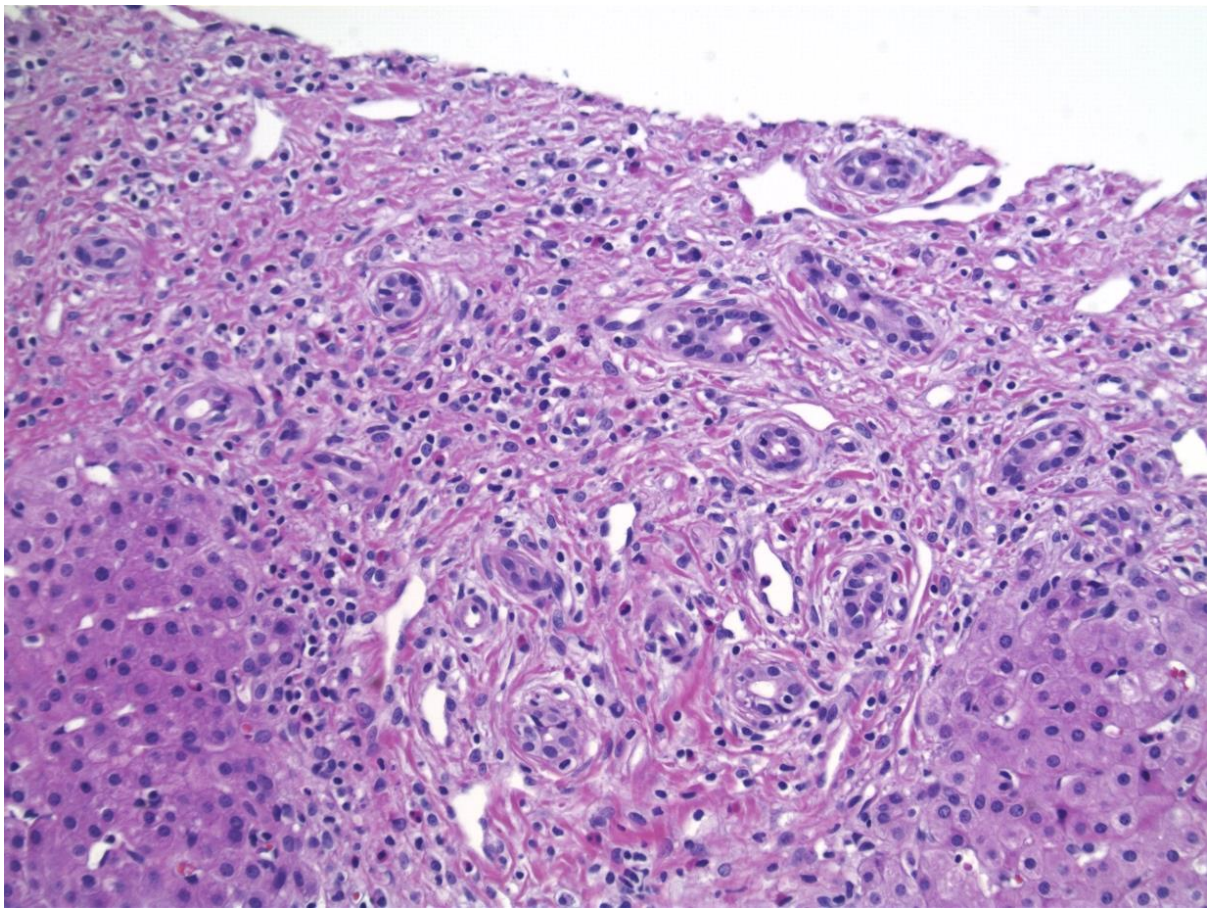


Figure 2

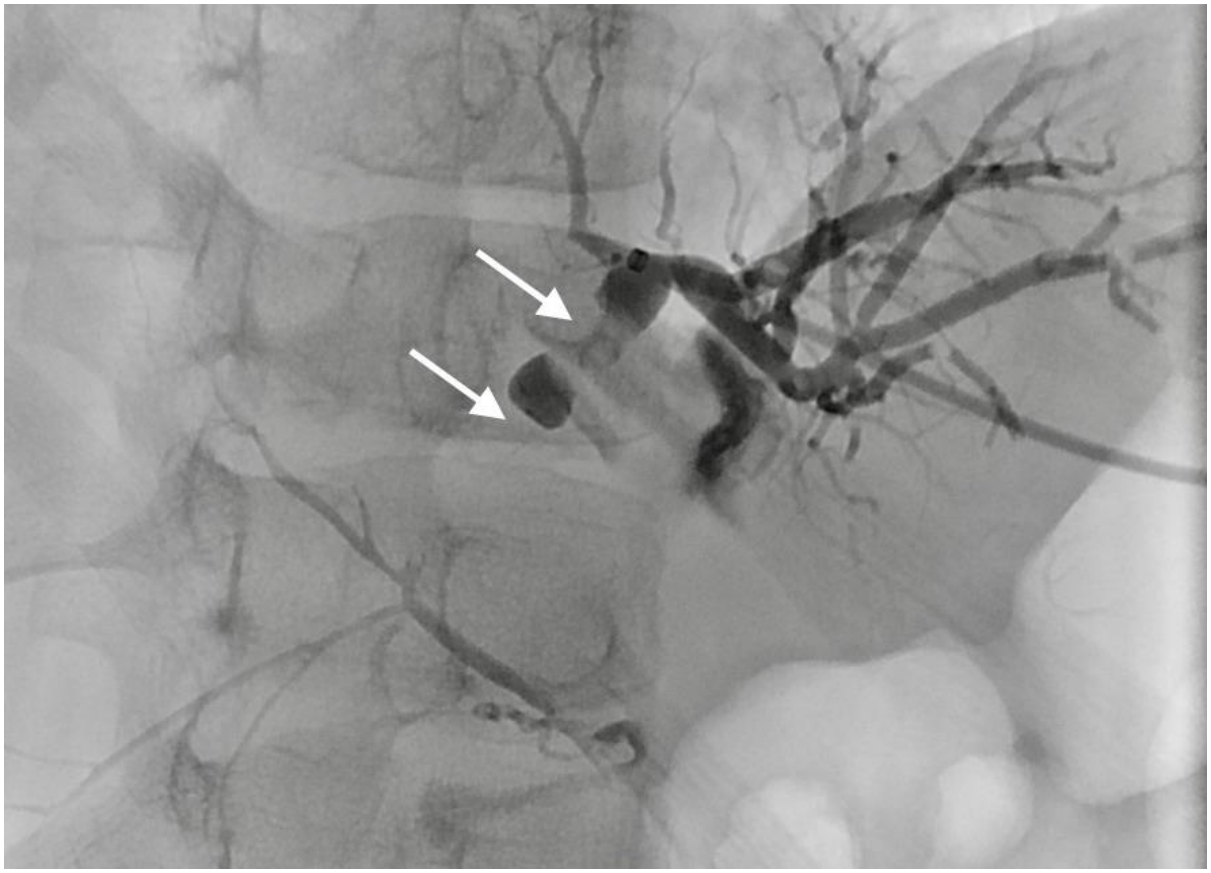


Figure 3A

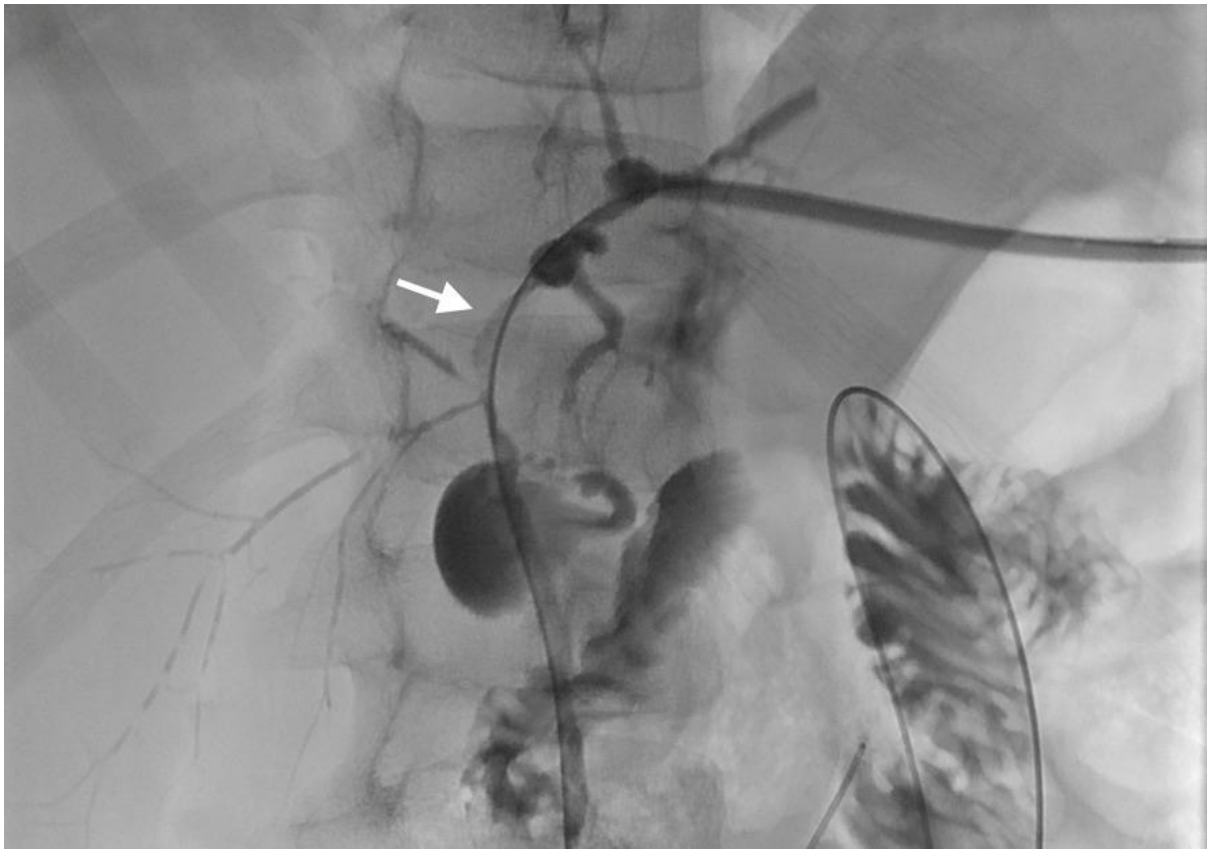


Figure 3B

3. New and Emerging Management Strategies

3.1. Couper MR, Valamparampil J, Thyagarajan M, Hartley J, Gupte G. Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation. Pediatr Transplant. 2023 Sep;27(6):e14574. doi: 10.1111/petr.14574. Epub 2023 Jul 17. PMID: 37458363.

Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation

Michael R Couper (1), Joseph Valamparampil (1), Manigan Thyagarajan (2), Jane Hartley (1), Girish Gupte (1)

Keywords –

Intestinal ultrasonography; Pediatric; Intestinal transplant

Affiliations –

1 – Liver Unit (including small bowel transplantation), Birmingham Women's, and Children's Hospital NHS Foundation Trust

2 – Radiology Department, Birmingham Women's, and Children's Hospital NHS Foundation Trust

Author contribution –

Michael Couper – Concept, manuscript writing, data collection and literature review

Joseph Valamparampil – Manuscript editing, expert opinion, patient recruitment

Manigan Thyagarajan – Expert opinion (Radiology), manuscript editing

Jane Hartley – Manuscript editing, expert opinion, patient recruitment

Girish Gupte – Concept, manuscript editing, expert opinion, patient recruitment

Corresponding author –

Girish Gupte

Consultant Paediatric Hepatologist

Liver Unit (including small bowel transplantation),

Birmingham Women's, and Children's Hospital NHS Foundation Trust

Steelhouse Lane, Birmingham B4 6NH

Tel no: +441213338255

Email: Girishgupte@nhs.net

Conflict of interest –

We have no conflicts of interest to declare.

We have no funding sources to declare.

Data availability statement –

The data underlying this article cannot be shared publicly due *to* the privacy of individuals (minors) participating in this study.

Consent –

Consent for inclusion and point of care testing was obtained from both parents and patients involved at time of examination. Written consent for inclusion of images was obtained from relevant families.

Abbreviations –

ACR – Acute cellular rejection

IUS – Intestinal ultrasound

Abstract

Background

Acute rejection is the leading cause of mortality and morbidity for children following intestinal transplantation. Rapid detection and prompt treatment are critical; however, the only reliable method of diagnosis and monitoring is endoscopic graft biopsies. The required regular anaesthetics are particularly problematic in children, and non-invasive strategies are needed.

Methods

We describe the intestinal ultrasound findings of three children before and after treatment for rejection. Ultrasounds were performed within 24 hours of endoscopically obtained biopsies which were used to establish a diagnosis of rejection and to define severity. A single sonographer performed the ultrasounds and was blinded to biopsy results at the time of the scanning. These findings are provided in the context of the ultrasound appearance of seven children who had no features of rejection on surveillance biopsies.

Results

Intestinal ultrasound demonstrated increased bowel wall thickness, vascularity, and mesenteric inflammation during moderate to severe rejection episodes. The submucosal layer was particularly thickened, which may represent a finding more specific for rejection. All patients demonstrated improvement in all quantitative ultrasound features correlating with the resolution of acute cellular rejection on histology. Patients with no evidence of rejection on biopsy had a bowel wall thickness range of 0.9 - 2.8 mm, suggesting a normal upper limit of 3 mm.

Conclusion

Moderate and severe acute rejection may be detected and response to treatment can be monitored by intestinal ultrasound and, correlating with clinical improvement, can aid in follow-up.

Introduction

Acute rejection is one of the leading causes of mortality and morbidity following intestinal transplantation affecting 50 – 75% of patients and is established on histopathology of biopsies from graft bowel[1]. Rapid detection and prompt treatment is important. The only reliable test to monitor treatment response is histopathology of graft biopsies. Requiring biopsies for diagnosis and assessment of treatment response is particularly problematic in children due to the frequent requirement for recurrent general anaesthetics. Additionally, the psychological impact on child and family cannot be underestimated. There is an unmet need for adjunctive diagnostic and monitoring tools in this patient group.

Intestinal ultrasound (IUS) is a promising modality for monitoring bowel disease. It is well tolerated, inexpensive, and does not require intravenous access or general anaesthesia[2]. While localisation following transplant can be challenging theoretically all or most of the graft can be assessed. It is well-established in monitoring paediatric inflammatory bowel disease, a group with similar high monitoring requirements by endoscopies and biopsies. Current ECCO-ESGAR guidelines recognise IUS as a potential alternative to endoscopy to assess treatment response[2]. There is limited literature on imaging features of acute cellular rejection in Intestinal transplant[3]. Diffuse wall thickening and mucosal hyper or hypo enhancement with ascites are reported to be suggestive[3,4]. Most reported experience is with computerised tomography and magnetic resonance imaging[5,10].

To date, no studies have investigated the role of point-of-care IUS in children with Intestinal transplant during acute cellular rejection (ACR) episodes. We report three patients who presented to our centre with acute rejection correlating IUS appearances with graft histology before and after treatment. We discuss our findings in the context of normal appearances based on IUS performed in seven children with no features of rejection on surveillance biopsies.

Method

In all cases, IUS were performed within 24 hours of the biopsies and before the histology results were available. All studies were performed within the same 6-month period. Consent for undergoing the point of care procedure and inclusion in the study was obtained from both parents and patient at time of examination.

Findings were documented per the classification systems utilised by the Gastroenterology network of intestinal ultrasound (GENIUS) and the International bowel ultrasound group (IBUS) (6). Recorded metrics included:

- Bowel wall thickness. Patients with a bowel wall thickness > 3 mm additionally had the separate bowel wall layers measured (mucosa, submucosa and muscularis).
- Bowel wall stratification. Loss of stratification between the bowel wall layers, hypoechoic or hyperechoic appearances.
- Vascularity. Scoring utilised the Limberg scoring system assessed using colour doppler. Limberg 0 normal vascularity, Limberg 1 short segments of blood vessels in the bowel wall, Limberg 2 long segments and pronounced blood vessels restricted to the bowel wall and Limberg 3 with long segments of blood vessels visible within the bowel wall with extension into the mesenterium.
- Mesenteric fibro-fatty proliferation. Score 0 no change in appearance; Score 1 increased hyperechoic appearance of the mesentery directly adjacent to inflamed bowel, and Score 3 diffuse hyperechoic fibro-fatty proliferation.
- Peristalsis. Scoring 2 normal peristalsis, scoring 1 sluggish peristalsis, and scoring 0 ileus.

No complications (stenosis, collections, fistula, or lymphadenopathy) were detected during the study.

Patient 1

An 8-year-old male with a previous isolated Intestinal transplant 6 years prior was admitted for fevers, vomiting, abdominal pain, and increased stool output. He had a background history of rejection 5 years prior and post-transplant lymphoproliferative disorder with treatment finishing 3 years prior. His immunosuppression regime consisted of Tacrolimus and Sirolimus. Enteric PCR was negative for infectious causes.

Histology demonstrated moderate ACR with apoptotic bodies in up to 8/10 crypts in both proximal and distal small bowel graft biopsies. IUS demonstrated graft bowel wall thickening (maximal 5.2 mm) with prominent stratification, increased vascularity (Limberg 3), mesenteric inflammation (scoring 2) and absent peristalsis (Figure 1). Native distal sigmoid colon was normal. He was treated with 3 separate courses of Methylprednisolone over the course of his admission.

Two weeks later, repeat graft biopsies demonstrated atrophy and regenerative changes. IUS demonstrated reduced bowel wall thickness (maximal 2 mm) with normal stratification, mild mesenteric inflammation (scoring 1) and improved but still sluggish peristalsis. Subsequent IUS demonstrated a return to normal asides reduced peristalsis, however, did not have matching histology for comparison. Patient clinically improved, was able to resume full enteral nutrition and is stable on follow-up.

Patient 2

A 16-year-old male was admitted for a multi-visceral transplant (Liver, stomach, small bowel, and pancreas) for hepatocellular carcinoma with portal vein involvement. His immunosuppression regime post-transplant consisted of Basaliximab, Methylprednisolone and Tacrolimus.

IUS performed on days 15 and day 21 post-transplant in conjunction with protocol post-transplant biopsies within normal limits had demonstrated bowel wall thickness < 3 mm, mildly increased vascularity (Limberg 1) and reduced peristalsis only.

He developed fever, increased stoma output and abdominal pain on day 36 and surveillance biopsies demonstrated severe ACR, with 14 apoptotic bodies seen in 10 consecutive crypts in distal small bowel graft biopsies. IUS demonstrated bowel wall thickness (maximal 6.9 mm) with prominent hyperechoic stratification, increased vascularity (Limberg 2), mesenteric inflammation (scoring 2) and absent peristalsis. Native distal sigmoid colon was normal. He was treated with pulse methylprednisolone and Basiliximab.

Repeat biopsies performed on day 80 post-transplant demonstrated no features of ACR. IUS demonstrated reduced bowel wall thickness (maximal 2 mm) with normal stratification, vascularity, and peristalsis. There was no mesenteric inflammation. Biopsies were performed in this interval demonstrating moderate then mild acute rejection; however, corresponding IUS is not available due to sonographer unavailability for this period.

Patient 3

A 13-year-old female with a previous Intestinal transplant 9 years prior was transferred for fever, vomiting and increased stool output with fresh blood mixed in. She had a background of previous episodes of rejection with the most recent episode 2 years 10 months prior. Her immunosuppression consisted of Tacrolimus, Sirolimus and Prednisolone. Enteric PCR was negative for infectious causes.

Histology demonstrated moderate ACR with up to 6 crypt base apoptotic bodies seen per 10 crypts in proximal graft colon and jejunum. IUS demonstrated bowel wall thickness (maximal 5.1 mm) with prominent stratification, increased vascularity (Limberg 3), mesenteric inflammation (scoring 2) and

sluggish peristalsis. Native sigmoid colon was normal. She was treated with a course of Methylprednisolone and due to lack of clinical improvement subsequently Anti-thymocyte globulin.

Repeat biopsies performed 8 days later demonstrated resolving ACR with no apoptotic bodies. IUS was not normal, with hypoechoic appearances of the bowel wall with a prominent submucosal layer. However, bowel wall thickness had improved (maximal 2.5 mm) with mild mesenteric inflammation (scoring 1) and no increased vascularity. Subsequent IUS two weeks following without corresponding histology demonstrated normal features with minimal peristalsis. Recovery was slow over several months however she returned to pre rejection baseline and was subsequently discharged.

Discussion

IUS in all the three Intestinal transplant recipients demonstrated increased bowel wall thickness, vascularity, and mesenteric inflammation during episodes of moderate to severe rejection. The bowel wall thickness was predominantly due to the expansion of the submucosal layer and may be a finding more specific to small bowel rejection (Table 1). The submucosal layer contains the arterial plexus with its arterioles branching into capillary networks to supply the mucosa and muscularis layers[7]. Our findings are consistent with previous large studies describing CT imaging findings post multivisceral transplantation. While CT appearances are nonspecific bowel wall thickening, mucosal hyperenhancement and ascites are common features[9]. This is the first study exploring appearances of ultrasound before and after treatment for acute rejection.

All three patients demonstrated improvement in all quantitative IUS features correlating with the resolution of ACR on histology. This is promising with potential utility in monitoring response to treatment in patients with an established diagnosis of acute rejection. As part of this study, seven Intestinal transplant recipients admitted for annual surveillance underwent IUS on the same day as having a biopsy taken. None had features of rejection on biopsy. This group's average maximal

bowel wall thickness was 1.54 mm (range 0.9 mm – 2.8 mm). While our study suggests that a cut-off of 3 mm, may be appropriate to diagnose rejection in the Intestinal transplant cohort, larger series are required to establish a more precise upper limit of normal in children[2, 6].

Larger studies in adult cohorts have previously reported bowel wall thickening > 5 mm as abnormal which is in line with an average of 5.7 mm (range 5.1 – 6.9 mm) found in our patients with rejection proven on histology[4].

Optimal timing of follow up ultrasound following commencement of treatment remains uncertain. This study prioritized pairing ultrasound studies with histology to appropriately correlate sonographic appearances. There was a wide range between the diagnostic study and subsequent study demonstrating improvement due to variability in timing of repeat biopsies. This however does represent clinical practice with studies finding episodes of acute rejection to vary in length correlating with severity (1 – 4 weeks)[11]. Patient 2 was approximately one-month post-transplant however as he had previously had two baseline ultrasound prior to his episode of rejection to establish a baseline it is reasonable to assume the changes then subsequent improvement on ultrasound were due to histologically confirmed rejection with subsequent improvement.

Previous studies investigating other imaging modalities to monitor Intestinal transplant have commented that it is challenging to establish the relevance of imaging features due to the small number of Intestinal transplant recipients[9]. More extensive studies are required to definitively define a correlation between imaging features and histology, particularly in mild rejection and subacute presentations. The role of IUS in patchy disease also remains unclear and the inability to pinpoint the exact location of disease in a graft is a weakness of the modality. Additionally, conventional sonography can be inherently limited by luminal gas precluding examination. In the authors experience it is uncommon for this to preclude assessment of the bowel wall. The bowel is situated proximally within the abdominal cavity unlike deeper viscera which are more prone to being

obscured. Larger studies in adult patients with Crohn's disease report an unsuccessful study rate for all indications of only 1.4%[10]. However, while examination was successful and good views were obtained in all studies bowel gas may also hinder the detection of patchy disease. Further data on sonographic appearances during viral enteritis and post-transplant lymphoproliferative disease are needed to establish if the observed sonographic features are specific to ACR. Despite its limitations, our study is prospective and demonstrates a clear sonographic distinction between cases with positive and negative histology for rejection. All studies were performed before histology results returned and within 24 hours of biopsies being obtained to minimise bias and ensure temporal correlation.

IUS has promise in children with Intestinal transplant during episodes of acute cellular rejection. Our study suggests that cases of moderate and severe ACR in Intestinal transplant may be detectable with IUS, and if detected on an initial study, improvement in response to treatment can be monitored and if correlating with clinical improvement, can aid in follow-up. A bowel wall thickness of 3 mm and prominent submucosal thickening may represent a finding in Intestinal transplant population more specific for rejection. Single or multi centre prospective trials of serial graft ultrasonographic surveillance may be warranted to further define the sensitivity and specificity of ultrasound in detection of various grades of graft rejection and discrimination of rejection from other graft pathologies.

References

1) Gürkan A. Advances in small bowel transplantation. Turk J Surg. 2017 Sep 1;33(3):135-141. doi: 10.5152/turkjsurg.2017.3544.
2) Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, Calabrese E, Baumgart DC, Bettenworth D, Borralho Nunes P, Burisch J, Castiglione F, Eliakim R, Ellul P, González-Lama Y, Gordon H, Halligan S, Katsanos K, Kopylov U, Kotze PG, Krustinš E, Laghi A, Limdi JK, Rieder F, Rimola J, Taylor SA, Tolan D, van Rheenen P, Verstockt B, Stoker J; European Crohn's and Colitis Organisation [ECCO] and the European Society of Gastrointestinal and Abdominal Radiology [ESGAR]. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. J Crohns Colitis. 2019 Feb 1;13(2):144-164. doi: 10.1093/ecco-jcc/jjy113
3) Hakim B, Myers DT, Williams TR, Nagai S, Bonnett J. Intestinal transplants: review of normal imaging appearance and complications. Br J Radiol. 2018 Oct;91(1090):20180173. doi: 10.1259/bjr.20180173. Epub 2018 Jun 5.
4) Sandrasegaran K, Lall C, Ramaswamy R, Redelman R, Hoff S, Rajesh A, et al.. Intestinal and multivisceral transplantation. Abdom Imaging 2011; 36: 382–9.
5) Khan N, Phillips GS, Heller MT, Linam LE, Parnell SE, Moshiri M, Bhargava P. Imaging in pediatric small bowel transplantation. Indian J Radiol Imaging. 2014 Oct;24(4):379-88.
6) Maren Hartmann, K. K. (2020). Compendium of Gastrointestinal Ultrasonography in Inflammatory Bowel Disease. Independent publication, TRUST group, 3 - 38.
7) Kvietys PR. The Gastrointestinal Circulation. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. Chapter 2, Anatomy. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53099/
8) Swanson BJ, Talmon GA, Wisecarver JW, Grant WJ, Radio SJ. Histologic analysis of chronic rejection in small bowel transplantation: mucosal and vascular alterations. Transplantation. 2013 Jan 27;95(2):378-82. doi: 10.1097/TP.0b013e318270f370.

9) Sandrasegaran K, Lall C, Ramaswamy R, Redelman R, Hoff S, Rajesh A, Vianna R. Intestinal and multivisceral transplantation. Abdom Imaging. 2011 Aug;36(4):382-9. doi: 10.1007/s00261-010-9680-y.

10) Kucharzik T, Wittig BM, Helwig U, Börner N, Rössler A, Rath S, Maaser C; TRUST study group. Use of Intestinal Ultrasound to Monitor Crohn's Disease Activity. Clin Gastroenterol Hepatol. 2017 Apr;15(4):535-542.e2. doi: 10.1016/j.cgh.2016.10.040. Epub 2016 Nov 14. PMID: 27856365.

11) Selvaggi G, Gaynor JJ, Moon J, Kato T, Thompson J, Nishida S, Levi D, Ruiz P, Cantwell P, Tzakis AG. Analysis of acute cellular rejection episodes in recipients of primary intestinal transplantation: a single center, 11-year experience. Am J Transplant. 2007 May;7(5):1249-57. doi: 10.1111/j.1600-6143.2007.01755.x. Epub 2007 Mar 15.

Patient	Symptoms	Time from transplant	Bowel wall thickness (mm)				Bowel wall stratification	Vascularity	Mesenteric inflammation	Peristalsis	Histology
			Total	Mucosa	Submucosa	Muscularis					
1	Fever, Diarrhoea, Vomiting, Pain	7 years	5.2	0.6	3.8 (76%)	0.6	Prominent	3	2	0	Acute rejection
	Feed intolerance	7 years, 1 month	2	-	-	-	Normal	0	1	1	Atrophy and regenerative changes
2	Fever, Diarrhoea, Pain	36 days	6.9	0.8	5.1 (76.2%)	0.8	Hyperechoic	2	2	0	Severe acute rejection
	Poor stoma output	77 days	2	-	-	-	Normal	0	0	2	No features of acute rejection seen
3	Fever, Diarrhoea, Pain	8 years, 11 months	5.1	0.7	3.4 (66.6%)	1	Prominent	3	2	1	Moderate acute rejection
	Diarrhoea, Electrolyte derangement	8 years, 11 months	2.5	-	-	-	Prominent	0	1	1	No features of acute rejection seen

Table 1 – Intestinal ultrasound features at time of diagnosis (light blue lines) and following treatment (white lines) with associated histology results.

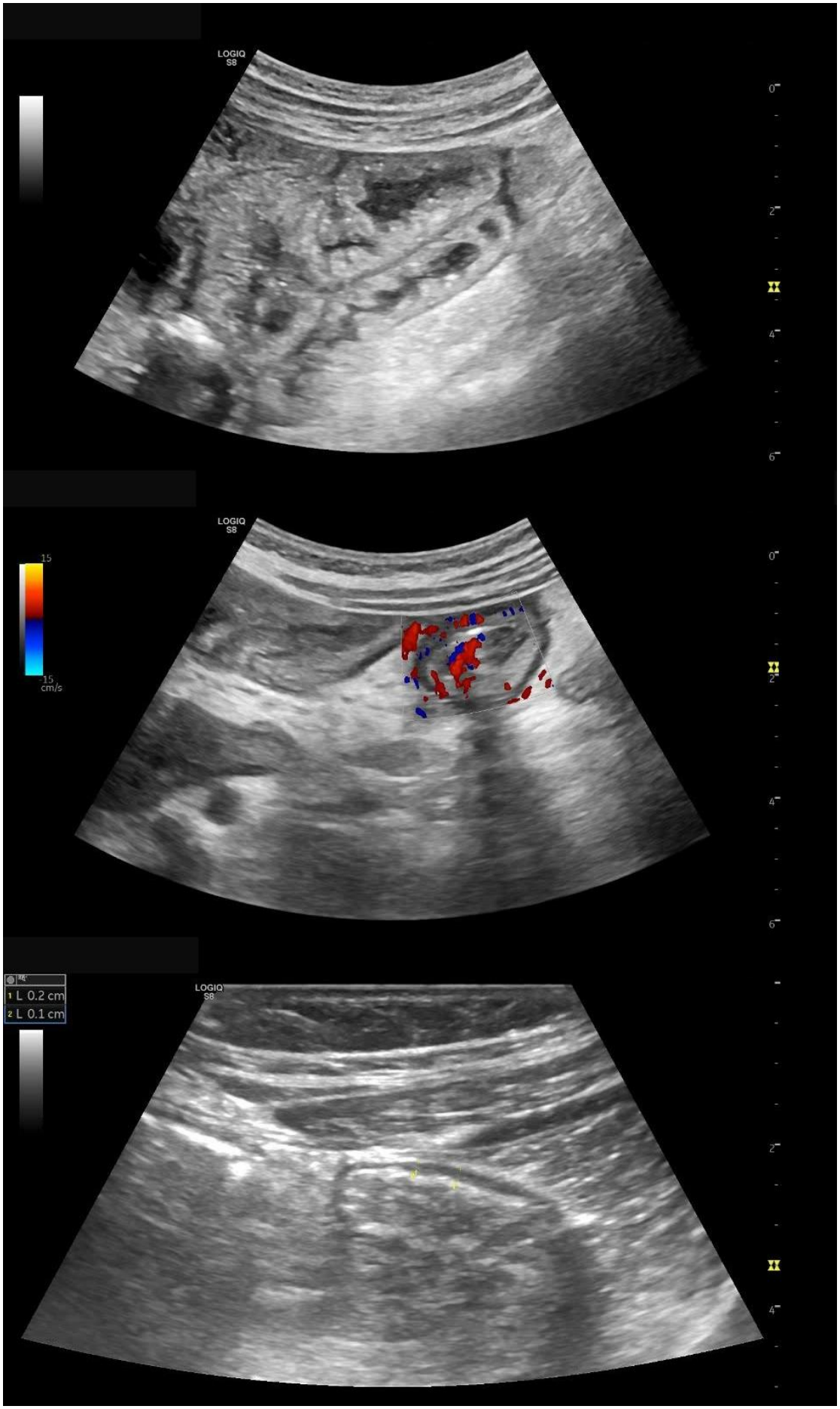

LOGIQ
S8
LOGIQ
S8
15
-15
cm/s
LOGIQ
S8
1 L 0.2 cm
2 L 0.1 cm

Figure 1 – IUS images from patient 1.

Top – Prominent loops of transplant graft with bowel wall thickening, prominent submucosal layer, and mesenteric inflammation.

Middle – Increased intestinal graft vascularity extending beyond the bowel wall (Limberg 3).

Bottom – Repeat study following treatment demonstrating resolution of bowel wall thickening.

3.2. Couper MR, Shun A, Siew S, O'Loughlin E, Thomas G, Andersen B, Jermyn V, Sawyer J, Stormon MO. Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021 Dec;25(8):e14092. doi: 10.1111/petr.14092. Epub 2021 Jul 27. PMID: 34313365.

Pediatric Third Liver transplantation – A single center experience

Authors -

M R Couper, S Siew, E V O'Loughlin, A Shun, G Thomas, B Andersen, V Jermyn, J Sawyer, M Stormon

Associations –

Children's Hospital at Westmead

Cnr Hawkesbury Rd &, Hainsworth St,

Westmead NSW 2145

Australian National Liver Transplant Unit

Royal Prince Alfred Hospital

Camperdown, Sydney

Abstract –

Pediatric retransplantation is accepted practice for graft failure and complications in Australasia. As 15% of children require a third transplant this is a growing cohort with currently limited data in the literature. We review nine patients from the commencement of our transplantation program in 1986 assessing demographics, prognosis and outcome measures. Post third transplant patient survival was comparative to first and second patient survival at five years. Third transplant operations were more complex, and all deaths were within the post-operative period. Neurological impairment and psychological disturbance appear to be prevalent and need to be considered in pre-transplant counselling.

What is known –

- Retransplantation is an accepted practice in children. Little data exists on third transplant patients.
- Several known poor prognostic factors are common and potentially cumulative in third transplant patients.
- Children post liver transplantation are at risk of neurological and renal impairment.

What is new –

- Patient survival rates post third transplant appear to be comparable to first and second transplant at 5 years.
- Third transplant is an operative challenge with on average twice the operative times and four times the transfusion volumes of initial transplants.
- These patients appear to have an extremely high rate of neurological impairment.

Introduction -

Liver retransplantation is the only corrective long-term management for graft failure and insurmountable graft complications. Previous single center cohorts have shown that 9% to 29% of pediatric liver transplantation patients need to undergo retransplantation[1]. In Australia since 2000 an additional 15% of children post retransplantation have required a third and possibly fourth transplants[2].

Pediatric retransplantation has previously been approached with caution. This is because of concerns about poorer graft and patient survival as well as a limited supply of donors[1,3,4]. The consensus opinion has however recently shifted. A 2020 retrospective cohort analysis of Australian and New Zealand liver transplant registry data demonstrated for children retransplanted between 2001-2017 actuarial graft survival of 84%, 75%, 70% and 54% and patient survival of 89%, 87%, 87% and 71% at 1 year, 5 years, 10 years and 15 years respectively[2]. This was not dissimilar to pooled survival rates for all Australasian children transplanted during that period with actuarial survival rates of 94%, 89%, 88% and 83% at 1 year, 5 years, 10 years and 15 years, respectively[5]. In addition, the pediatric wait list mortality for Australia and New Zealand in 2017 was 1.4%. An intention since 2002 to split livers wherever possible and improvements in organ donation have played a large role in achieving this[5].

Because of lack of treatment alternatives, improved outcomes and low wait list mortality the current practice in Australia and New Zealand is to undertake liver retransplantation in children. The decision to undertake a third pediatric liver transplant is more contentious, hence this retrospective cohort study reporting our single center experience in these patients.

Methods –

All patients who had a third liver transplant at The Children's Hospital Westmead, Sydney, Australia since the transplant program started in 1986 are reported. Patient files were reviewed and age at first transplant, time frame between transplants, gender, initial transplant indication, indications for retransplant, graft types, immunosuppression used, episodes of acute rejection following each transplant, pediatric end-stage liver disease (PELD) / model for end-stage liver disease (MELD) scores, pre transplant renal function, renal function 1 year post third transplant, operative issues, warm ischemia times, operative times, post-operative complications, intraoperative transfusion volumes, neurocognitive outcomes and mortality are reported. Renal function was calculated using eGFR. Blood volumes at transplant as a percentage of patient total estimate blood volume were calculated utilizing patient weight at time of transplant and red cross estimated ml/kg blood volumes by age group[17]. Neurocognitive outcomes include any diagnosis or reports of cognitive delay, motor impairment, academic accommodations / issues and psychiatric diagnosis were recorded. Some data was missing particularly for patients undergoing transplant before 2000 in part because of electronic medical record complexities.

Results –

Nine patients underwent a third transplants (3 F : 6 M). Five patients had an initial diagnosis of extrahepatic biliary atresia, two with progressive familial intrahepatic cholestasis syndrome type 1, one infantile cholestasis of unknown etiology and one cryptogenic cirrhosis. Graft type for third transplant was predominantly whole (n = 7). The remaining two received a living related donor

segment 2/3 split graft and a reduced graft, respectively. Two had their third transplant in the 1990s, four in the 2000s and three in the 2010s (table 1). Immunosuppression at the time of third liver transplant correlated with the era of transplant: patients in 1990's received glucocorticoids and cyclosporin with or without azathioprine, patients in the 2000's received primarily glucocorticoids, azathioprine and tacrolimus, while patients from 2010's received glucocorticoids, basiliximab and tacrolimus.

Three of the nine patients (33%) died. This included the two patients from the 1990s and a patient in the 2010s. All three succumbed to sepsis with subsequent multiorgan failure within the post-operative period. The remaining six patients are alive with minimum follow up being four years (mean 11 years, range 4 – 18 years) post third liver transplant (graph 1).

Operative times greatly increased with each retransplant. Mean operating times for initial, second and third liver transplant were 8 hours 42 minutes, 9 hours 32 minutes and 15 hours 50 minutes, respectively (graph 2). Similarly, cold ischemic times increased with subsequent transplant with initial, second and third liver transplant times of 8 hours 38 minutes, 9 hours 25 minutes and 12 hours 7 minutes respectively. This trend was despite one third transplant who had a living related donor resulting in a substantially lower than average ischemic times. Warm ischemia times were not substantially different. The volume of blood products required significantly increased by the third transplant, with average transfusion volumes at initial, second and third transplant 1623 ml, 2921 ml and 6713 ml, respectively (table 1). This did not change when adjusted to correct for patient estimated total blood volume based on age and weight at time of each transplant (total transfusion volumes as percentage of total estimated blood volume, 144%, 181% and 316% respectively). There was no substantial difference in average operative times or adjusted transfusion volumes in survivors compared to deceased patients.

There was no correlation between third transplant PELD / MELD scores, operative times, blood losses or mortality. This is not surprising given PELD / MELD scores main utility is in assessing mortality risk while awaiting transplant and not for post-transplant prognosis.

One patient following third transplant developed renal failure with a diagnosis of membranoproliferative glomerulonephritis secondary to calcineurin toxicity. This was evident within one-year post third transplant with an eGFR = 75 ml/min/1.73m2. This patient subsequently went on to have a successful renal transplant after a period of dialysis. All pretransplant eGFRs were normal (normal eGFR = 125 ml/min/1.73m2)[6] and all eGFRs one-year post third transplant, other than the aforementioned patient, were also normal.

Of the 6 third transplant survivors, 5 (83%) were reported to have learning difficulties, with two requiring special education, one requiring a reduced academic load and one home schooling. Psychological issues were common affecting four out of six survivors (66.6%) with diagnoses of anxiety, behavioral issues, depression and night terrors (table 1).

Discussion –

Performing a third liver transplant on a child is a major undertaking for not only the child and their family, but for the whole transplant team. This case series over a 30 plus year period highlights some of the issues encountered in 9 patients undergoing a third liver transplant at our institution, adding to the small body of literature on this topic.

The overall survival rate for children in this small cohort was 66%, which improves to 86% if restricted to the most recent era of 2001-2017. This compares favorably with the recent report of the Australian and New Zealand experience of second transplants in children, with 5- and 10-year survival of 87%[2]. Most retransplant case series are based on second liver transplants, although a 2007 paper from Chile described five children post third transplant with a 60% survival rate at 1 and 5 years[7].

Various factors are reported to affect outcomes in children undergoing retransplantation. Some studies demonstrate a poorer prognosis for patients on life support at retransplantation, for split graft recipients, and for patients transplanted because of neonatal or familial cholestasis / paucity of bile ducts / congenital abnormalities[1,4,8]. Subsequent studies found no difference between split, reduced and whole liver grafting[2,3]. Not surprisingly it's been shown that worse outcomes after a second liver transplant are due in part to children being in poorer clinical condition pre-transplant[4]. In our study no patients were on life support or in ICU prior to their third transplant, apart from one instance of primary non function at day 2 after that patient's second transplant.

All three deaths post-transplant were within the immediate post-operative period, with no further mortality with a minimum of 4 years follow up. This suggests the main risk period is within the first 3 months post-transplant[2], which is further supported by the increased transfusion volumes and operative times reflecting the tremendous insult of such major surgery. Volumes of blood products and length of surgery were on average, respectively, more than 4 times and nearly 2 times higher when compared to patients' initial and second transplant. In keeping with longer operative times average cold ischemic times also increased with subsequent transplants. The total volume of blood transfused / blood loss at surgery, along with warm ischemia times > 60 minutes, and, longer cold ischemia times particularly > 12 hours have been reported previously to affect outcomes[3,4,8,19]. These factors result in poorer donor organ preservation increasing the risk of allograft dysfunction. Several factors contribute to third transplant operations being uniquely difficult. We found that dense adhesions, distorted liver vasculature and biliary-enteric anatomy from previous transplants complicated removal of the explant and insertion of the new donor organ. This patient group is also uniquely at risk for hemorrhage as indicated by increased transfusion volumes. Neo-vascularization within adhesive bands and portal hypertension in the presence of coagulopathy are common. This is particularly evident in patients who have received a partial graft or whose graft failed secondary to hepatic artery thrombosis due to diffuse graft arterialization via collaterals. Improvements in surgical technique and hemostatic instrumentation help explain the improvement in outcomes since the 1990s for third transplantation.

All 3 patients who died had undergone transplant for chronic rejection, 2 of whom died prior to 2000 in an era before contemporary immunosuppression regimes. Additionally, UW cold storage solution while introduced in the US in 1987 was not available in Australia until the mid-1990's impacting preservation of donor organs prior to transplantation. As both our patients who underwent third transplant did so in the early 1990s simple cold storage may have increased the risk of graft dysfunction, rejection and, due to higher immunosuppression requirements, increased the risk of infection. Contrary to our findings previous studies have suggested better retransplantation outcomes when rejection was the indication, with reports of a 44% reduced risk of mortality[1]. The rest of the cohort was retransplanted for a mix of primary non function, vascular and biliary complications.

The era of transplant has a significant effect on outcomes, and undoubtedly the steady advances in medical and surgical management which have substantially improved pediatric liver transplantation over the last three decades are especially relevant for outcomes after retransplantation. Improvements across multiple areas impact on results – immunosuppressive regimes, surgical

expertise, preservation solutions and recipient and donor selection among them. Organ donation rates in Australia have improved over the past decade[18], with subsequent improvements in wait list times resulting in improved pre-transplant clinical condition.

Transplant patients are specifically at risk of long-term renal disease due to a combination of their underlying disease process, potential hepatorenal syndrome, early post-transplant fluid balance issues, procedures with extended caval cross clamping times and renal toxicity from CNIs[9]. Studies of adult post-transplant patients suggest a 2 – 5% risk per year of renal dysfunction[10], while studies of post-transplant renal function in children have shown that up to 20% develop chronic kidney disease, with eGFR < 90ml/minute/1.73m2 [3]. This is reflected in one of the six survivors in this cohort who ultimately underwent a renal transplant. This experience has resulted in our institution transplant protocol adopting a renal sparing immunosuppressive regime for third transplantations after 2010. The prolonged operative time and large transfusion volumes do suggest an increased risk in this group of complex retransplant procedures, as demonstrated by one study of adult transplant patients which found a 4% increase in risk for lack of renal recovery per minute of warm ischemia perfusion time (P=0.04)[11].

Neurodevelopmental and psychological performance are important outcome measures for children after liver transplant. Although formal psychometric testing was not routinely undertaken, our cohort demonstrated high levels of learning difficulties and psychological issues post third liver transplant. Multiple factors interfere with healthy neurocognitive development: severe underlying liver disease with infection, hyperbilirubinemia, hepatic encephalopathy and malnutrition; exposure to drugs such as glucocorticoids and CNIs[12]; and, prolonged hospitalization and anesthesia. Depending on when and how these deficits are measured, multiple publications report variable post-transplant outcomes: cognitive delays (18%), learning disability (26%), a 16% rate of anxiety or post-traumatic stress disorder, and 5% with a severe psychiatric disorder[12,13,14,15]. One study reported 34% of post-transplant children required special educational services[14], with average post-transplant cognitive abilities in the low-average range (FSIQ < 70 in 7.5% and a further 25% borderline 70-84)[16]. Afshar et al described poorer neurocognitive outcomes in children post-transplant for chronic liver disease with increased time spend on wait list and older age at transplant[16]. Given these factors are feasibly cumulative our results are perhaps unsurprising. Our small cohort of 6 survivors had significant learning difficulties (>80%) and psychological issues (nearly 70%), which are very important factors to consider when deciding whether a child should undergo a third liver transplant.

Conclusion –

Our study has several limitations. It is a small, retrospective and highly selected group of patients chosen to undergo a third transplant. The small numbers of patients over such a long period of time, impacted by improvements in liver transplantation over the eras of treatment, makes it difficult to draw firm conclusions about this undertaking. Whilst most recent mortality outcomes are comparable to those undergoing a second liver transplant, the long-term neurodevelopmental outcomes can be sobering and demand careful family counselling prior to undertaking a third transplant.

References -

1 – Davis A, Rosenthal P, Glidden D. Pediatric liver retransplantation: outcomes and a prognostic scoring tool. Liver Transpl. 2009 Feb;15(2):199-207. doi: 10.1002/lt.21664. PMID: 19177452.

2- Jeffrey AW, Jeffrey GP, Stormon M, Thomas G, O'Loughlin E, Shun A, Hardikar W, Jones R, McCall J, Evans H, Starkey G, Hodgkinson P, Ee LC, Moore D, Mews C, McCaughan GW, Angus PW, Wigg AJ, Crawford M, Fawcett J. Outcomes for children after second liver transplantations are similar to those after first transplantations: a binational registry analysis. Med J Aust. 2020 Nov;213(10):464-470. doi: 10.5694/mja2.50802. Epub 2020 Oct 4. PMID: 33015834.

3 – Venick RS, Farmer DG, Soto JR, Vargas J, Yersiz H, Kaldas FM, Agopian VG, Hiatt JR, McDiarmid SV, Busuttil RW. One Thousand Pediatric Liver Transplants During Thirty Years: Lessons Learned. J Am Coll Surg. 2018 Apr;226(4):355-366. doi: 10.1016/j.jamcollsurg.2017.12.042. Epub 2018 Feb 2. PMID: 29410290.

4 – Sieders E, Peeters P, TenVergert E, De Jong K, Porte R, Zwaveling J, Bijleveld C, Scloof M. Retransplantation of the liver in Children. Transplantation. 2001 Jan 15;71(1):90-95

5 - Australia and New Zealand Liver transplant registry. 30th annual ANZLITR report. Report on Liver and intestinal transplantation activity to 31-12-2019. 2020.

6 – Chapter 1: Definition and classification of CKD. Kidney Int Suppl (2011). 2013 Jan;3(1):19-62. doi: 10.1038/kisup.2012.64. PMID: 25018975; PMCID: PMC4089693.

7 – Uribe M, Buckel E, Ferrario M, Hunter B, Godoy J, González G, Cavallieri S, Iñiguez R, Calabrán L, Herzog C. Pediatric liver retransplantation: indications and outcome. Transplant Proc. 2007 Apr;39(3):609-11. doi: 10.1016/j.transproceed.2006.12.031. PMID: 17445556.

8 – Dreyzin A, Lunz J, Venkat V, Martin L, Bond GJ, Soltys KA, Sindhi R, Mazariegos GV. Long-term outcomes and predictors in pediatric liver retransplantation. Pediatr Transplant. 2015 Dec;19(8):866-74. doi: 10.1111/petr.12588. Epub 2015 Sep 12. PMID: 26362966.

9 – Isa HM, Mohamed AM, Alderazi AE. Effect of pediatric liver transplantation on renal function. Saudi J Kidney Dis Transpl. 2016 Jan;27(1):1-8. doi: 10.4103/1319-2442.174041. PMID: 26787559.

10 – Parajuli S, Foley D, Djamali A, Mandelbrot D. Renal Function and Transplantation in Liver Disease. Transplantation. 2015 Sep;99(9):1756-64. doi: 10.1097/TP.0000000000000820. PMID: 26308413.

11 – Laskey HL, Schomaker N, Hung KW, Asrani SK, Jennings L, Nydam TL, Gralla J, Wiseman A, Rosen HR, Biggins SW. Predicting renal recovery after liver transplant with severe pretransplant subacute kidney injury: The impact of warm ischemia time. Liver Transpl. 2016 Aug;22(8):1085-91. doi: 10.1002/lt.24488. Epub 2016 Jul 12. PMID: 27302834.

12 – Rodijk LH, den Heijer AE, Hulscher JBF, Verkade HJ, de Kleine RHJ, Bruggink JLM. Neurodevelopmental Outcomes in Children With Liver Diseases: a Systematic Review. J Pediatr Gastroenterol Nutr. 2018 Aug;67(2):157-168. doi: 10.1097/MPG.0000000000001981. PMID: 29601439.

13- Martinelli J, Habes D, Majed L, Guettier C, Gonzalès E, Linglart A, Larue C, Furlan V, Pariente D, Baujard C, Branchereau S, Gauthier F, Jacquemin E, Bernard O. Long-term outcome of liver transplantation in childhood: A study of 20-year survivors. Am J Transplant. 2018 Jul;18(7):1680-1689. doi: 10.1111/ajt.14626. Epub 2018 Jan 12. PMID: 29247469.

14 – Gilmour SM, Sorensen LG, Anand R, Yin W, Alonso EM; SPLIT Research Consortium. School outcomes in children registered in the studies for pediatric liver transplant (SPLIT) consortium. Liver Transpl. 2010 Sep;16(9):1041-8. doi: 10.1002/lt.22120. PMID: 20818741; PMCID: PMC2936718.

15 – Capone K, Amirikian K, Azzam RK. Pediatric Liver Transplantation: An Update for the Pediatrician. Pediatr Ann. 2016 Dec 1;45(12):e439-e445. doi: 10.3928/19382359-20161121-01. PMID: 27975113.

16 - Afshar S, Porter M, Barton B, Stormon M. Intellectual and academic outcomes after pediatric liver transplantation: Relationship with transplant-related factors. Am J Transplant. 2018 Sep;18(9):2229-2237. doi: 10.1111/ajt.14924. Epub 2018 Jun 16. PMID: 29745028.

17 - Australian Red Cross, Haemorrhage management. https://transfusion.com.au/disease_therapeutics/haemorrhage. Last accessed 12-02-2021.

18 - McCaughan GW, Munn SR. Liver transplantation in Australia and New Zealand. Liver Transpl. 2016 Jun;22(6):830-8. doi: 10.1002/lt.24446. PMID: 27028552.

19 - Sibulesky L, Li M, Hansen RN, Dick AA, Montenovo MI, Rayhill SC, Bakthavatsalam R, Reyes JD. Impact of Cold Ischemia Time on Outcomes of Liver Transplantation: A Single Center Experience. Ann Transplant. 2016 Mar 8;21:145-51. doi: 10.12659/aot.896190. PMID: 26952540.

Third retransplant decade	Number	Gender		3rd transplant graft type			Mortality	Average operative times			Average transfusion volumes (ml)			Average Ischaemic times, Cold:Warm (mins)		
		Male	Female	Split	Whole	Reduced		First	Second	Third	First	Second	Third	First	Second	Third
Before 2000	2	1	1	0	2	0	2/2 (100%)	8 hours 16 minutes	7 hours 46 minutes	10 hours 34 minutes	2380	5710	4955	N/A	N/A	N/A
2000 - 2010	4	3	1	1	3	0	0/4 (0%)	9 hours 43 minutes	12 hours 22 minutes	18 hours 40 minutes	1936	3836	4956	486:69	693:68	789:56
2010 - 2020	3	2	1	0	2	1	1/3 (33.3%)	8 hours	8 hours 49 minutes	16 hour 33 minutes	805	956	8554	550:46	438:57	645:66
Combined	9	6	3	1	7	1	3/9 (33.3%)	8 hours 42 minutes	9 hours 32 minutes	15 hours 50 minutes	1623	2921	6713	518:58	565:62	727:59

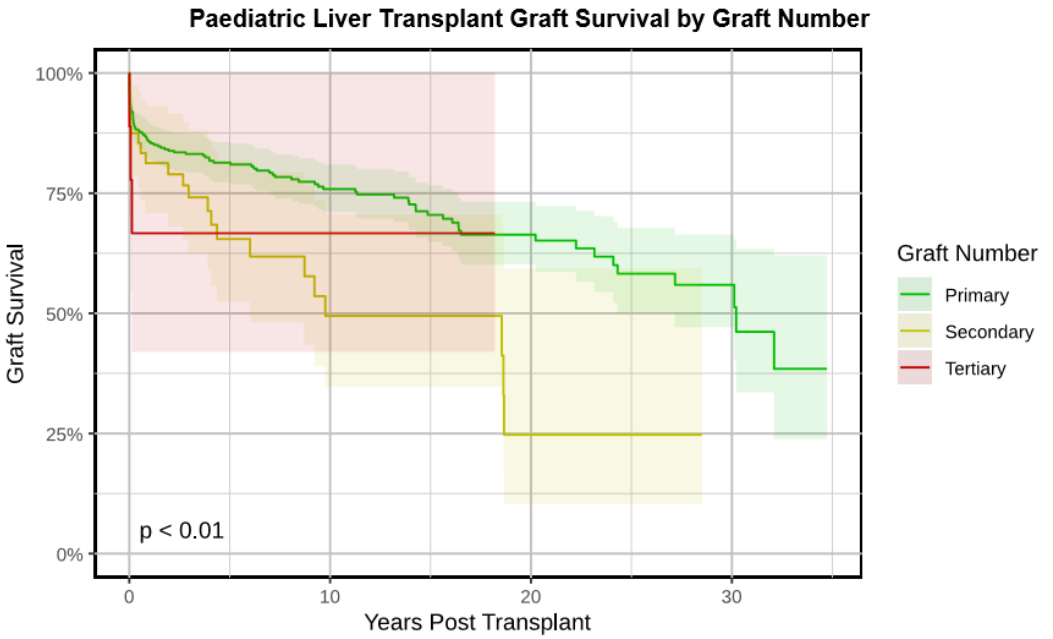
Table 1

- Patient characteristics and outcome measures whole cohort and by decade of third transplant
- Ischemic times for patients before 2000 was not available

Third retransplant decade	Post 3rd transplant renal dysfunction (eGFR < 90 ml/min/1.73m2)	Cognitive delay post 3rd transplant	Post 3rd transplant psychological diagnosis
Before 2000	0/2 (0%)*	1/2 (50%)*	0/2 (0%)*
2000 - 2010	1/4 (25%)	3/4 (75%)	2/4 (50%)
2010 - 2020	0/2 (0%)	2/2 (100%)	2/2 (100%)
Combined	1/6 (16.6%)	5/6 (83%)	4/6 (66.66%)

Table 2

- Secondary outcome measures whole cohort and by decade of third transplant
- * Data is pre 3rd transplant due to no survivors beyond post-operative stage following 3rd transplant in this group. Not included in combined totals.



Graft	Years Post-Tx	Num at Risk	Survival
Primary	0	359	100 %
Primary	1	289	86 %
Primary	2	267	84 %
Primary	5	208	81 %
Primary	10	146	76 %
Primary	20	54	66 %
Primary	34	1	38 %

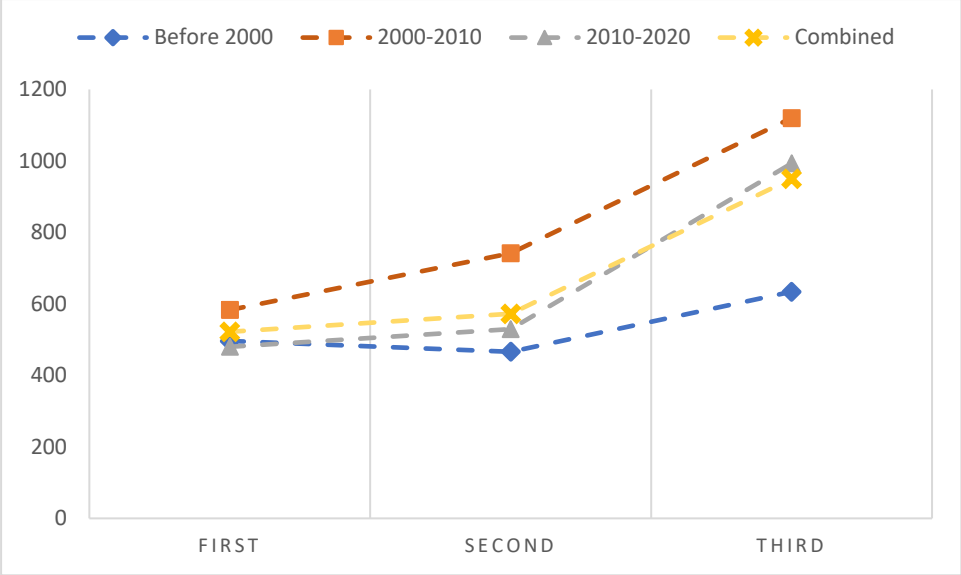
Graft	Years Post-Tx	Num at Risk	Survival
Secondary	0	48	100 %
Secondary	1	39	81 %
Secondary	2	34	79 %
Secondary	5	19	65 %
Secondary	10	12	49 %
Secondary	20	2	25 %

Graft	Years Post-Tx	Num at Risk	Survival
Tertiary	0	9	100 %
Tertiary	1	6	67 %
Tertiary	2	6	67 %
Tertiary	5	5	67 %
Tertiary	10	4	67 %

Graft survival not censored
Includes patients of paediatric age (<16 yr) at time of transplant

Graph 1

- Post-transplant graft survival for Pediatric (<16 year) patients for 1st, 2nd and 3rd transplant. 95% confidence intervals calculated via Kaplan-Meier estimate represented by adjacent shading.



Graph 2

- Average operative times in minutes for 1st, 2nd and 3rd transplant by transplant generation and combined total cohort.

CHAPTER 3 – LIMITATIONS, FURTHER DIRECTION, AND CONCLUSIONS

1. Limitations

This chapter aims to further explore limitations of this thesis and its included works. This thesis covers a broad topic highlighting advancements in recognition and management of novel and rare conditions, ongoing areas of challenge and advancements in transplantation management encountered in the author's career. Without some brevity this topic cannot be covered in its entirety. Instead, this thesis attempts to provide an overview on the subject with discussion guided by the authors research and clinical practice in large paediatric tertiary centres over the last decade.

The included publications have several limitations inherent to the underlying investigations and limited sample sizes. Publications 1.2, 1.3, 2.1, 2.2 and 3.2 are retrospective in nature and have small, often single digit patient populations. The insights provided are limited by the assumption that the original data as recorded and available in the records is valid and reliable. Additionally, the smaller sample sizes introduce the possibility of outlier effects skewing result interpretation. This does however represent a challenge in general in studying these population groups. Several of the conditions discussed in this thesis are either novel or have an extremely low incidence and prevalence. Such research is necessary to allow a body of evidence to develop in the literature to facilitate meaningful conclusions as well as provide guidance to the clinical gastroenterologist. Additionally, it is often better for novel research hypothesis to be tested or for trends to be identified in a small number of subjects to determine whether resources should be allocated to larger more robust studies.

Publication Appendix 1.1 (*Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics, 2024*) is a large retrospective cohort study with a study period over several decades. This introduces limitations and challenges. In some cases, historical data was not available limiting evaluation. Additionally, over the study period definitions of

the various types of failure such as acute kidney injury have changed making direct comparison between study periods challenging as well as establishing which prognostic factors are more important. Similarly diagnostic criteria change makes the comparison of diagnosis fraught. A prime example is the historical diagnosis of neonatal hemochromatosis which in many cases would have been used to described patients with Gestational alloimmune liver disease (GALD). Contemporarily GALD has a much stricter diagnostic criteria and neonatal hemochromatosis / hepatic siderosis is seen as a finding in a number of liver diseases and as such is not as a specific diagnosis. In already rare conditions requiring longer study periods to generate an appropriately powered cohort these limitations are amplified. As discussed, the increasing incidence of neonatal liver failure as well as contributors for certain diagnostic groups may relate to improving diagnostic criteria and tests, rather than a true increase in incidence in these conditions.

2. Further directions

Covering a large component of clinical Paediatric Gastroenterology, the future directions for research for the topics covered within this thesis are protean. This is particularly demonstrated by Appendix 1 *Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics. 2024* published following the work in the body of this thesis and demonstrating the paradigm shift in diagnosis, outcomes, and management of neonates with liver failure over the last thirty years. While touched on in the conceptual statements in individual chapters this section aims to expand on future directions and current areas of emerging research interest within the specialty –

2.1. Organoid Research

Organoids are defined as a 3D cell culture system that mimics the structure and function of the represented organ. Liver hepatocyte organoids have been created with differentiation of pluripotent stem cells in 2013 and from tissue specific progenitor cells which preserve the regenerative capacities of the organ in 2015 ((106,107). Additionally specific cholangiocyte organoids have been grown since 2014 to allow specific study of biliary systems (108).

Organoid research has had numerous applications to date and has great potential for the future directions of multiple topics and themes explored in this thesis. Disease specific organoids to mimic the liver for multiple different liver diseases have been created (Table 7 – Liver disease specific organoids, page 215 (109))

This has provided an avenue within these conditions to explore both the underlying pathogenesis of these diseases as well as to tests potential therapies. Additionally, this approach has promise in genetic conditions to trial gene therapy and gene editing approaches. This has been demonstrated in organoid models of Citrullinemia type 1 with organoids mimicking the clinical phenotypes observed in patients as well as demonstrating that these models could be rescued with ectopic expression of the wild type Arginosuccinate Synthase 1 ASS1 gene, the enzyme deficiency that results in this condition (109). Similar models have been used to investigate predominantly biliary diseases such as Cystic fibrosis with CFTR mutation specific drugs testing in patient derived organoids. Host-microbiome interactions in Hepatitis C disease have been explored with organoids (109). Organoids have also been utilised for toxicity screening of new medications as well as exploring cytochrome P450 mediated toxicity mechanisms (110). Organoid technology could be utilised for the study and treatment of rare diseases and novel infectious processes such as those described in publication 1.1, *Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med. 2022* and 1.2 *Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023*. It has promise in areas of need and has already been used to investigate DILI and biliary disease similar to those discussed in publications 2.1, *Periportal*

necrosis and successful liver transplantation following Lamotrigine drug induced liver injury in a child. BMJ case reports. 2023 and 2.2 *Hepatobiliary and Pancreatic: A rare peribiliary lesion. J Gastroenterol Hepatol. 2021*. Particularly in DILI, future patient specific drug screening with patient specific organoids, while unlikely to be feasible in the near future, has great potential. It would allow high throughput screening of potential therapeutic agents from biobanks. Models of specific hepatic cancers have also been created to research underlying pathogenesis and trial treatments – a promising future direction for the topics discussed in publication 1.3, *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr. 2021.*

There has also been interest in using functional hepatic organoids in liver transplantation. With the theoretical possibility of being able to generate an HLA-matched tissue specific this holds great promise for reducing the risk of common causes of morbidity and graft failure such as rejection. Early work in this area has been seen with reports of functional engraftment of orthotopically transplanted organoids in the liver (106,107,111,112). This clearly holds great promise for the future of the topics raised in publications 3.2 *Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021* and 3.1 *Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation. Pediatr Transplantat 2023*. Similarly for metabolic conditions where a small percentage of native enzyme activity may abolish adverse consequence, organoid engraftment is an exciting prospect.

2.2. Gene therapy

Liver transplantation is a growing indication for many inherited metabolic diseases. A graft allows for replacement of the deficient enzyme. This therapy is imprecise in that it replaces all the recipient's

liver enzymes, which are functioning normally, with those of the donor in order to correct a single deficit. Additionally, it comes at the cost of life long immunosuppression and any post-transplant complications. With increased understanding of the underlying cause of these conditions directly correcting the underlying cause is highly appealing with corresponding interest in liver directed gene therapy.

Gene therapies typically rely on adeno-associated viral (AAV) vectors as typically these are non-pathogenic and are replication deficient relying on co-infection helper viruses to replicate. These have the added benefit of relatively low immunogenicity. Recombinant AAV vectors are designed to contain a transgene expression cassette allowing delivery of a therapeutic transgene to the hepatocyte nucleus for long term expression. The liver is a particularly promising target given its relative immunotolerance (113). Several phase I and II trials are currently underway for many conditions (Table 8 – Liver directed gene therapy clinical trials, page 216 (113))

Gene therapy has limitations and several challenges to overcome. The main challenge is side effects and ineffective therapy due to immune response triggered by the capsid, vector genome and the protein product of the transgene. This is a particular problem if the defect is caused by a null mutation and the patient is naïve to the protein. Hepatotoxicity, Neurotoxicity and complement activation have also been reported limiting therapy in recipients. Despite these challenges this remains a promising area particularly in relation to the topics discussed in publication 1.2, *Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023* and Appendix 1, *Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics 2024.*

2.3. Fluorescence-guided liver surgery

As discussed in publication 1.3 outcomes post resection for Inflammatory Myofibroblastic tumours as well as other localised liver lesions is typically excellent when full resection is achieved. Incomplete resection can lead to local recurrence, further morbidity and depending on the lesion mortality. For example, Hepatocellular carcinoma may be cured with complete resection however residual disease results in a 5-year recurrence rate of 70% (114).

Recent studies have explored the use of fluorescence image guided surgery on HCC tumour-bearing mice. Mice were injected with a targeted small molecule fluorescent probe 8 hours before tumour resection surgery. Using near-infrared II imaging during surgery, tumours could easily be distinguished from other organs down to areas 2 mm in diameter with tumour confirmed on histology.

While in early stages this holds a lot of promise for the topics discussed in publication 1.3, *Paediatric Gastrointestinal, Hepatic and Pancreatic Inflammatory Myofibroblastic Tumours, A Single Centre Experience. J Pediatr Gastr. 2021* with the potential to increase rates of cure by complete resection and reduce disease recurrence (114).

2.4. Alternate transplant techniques

Since the introduction of intention to split policies in Australia paediatric liver transplantation has predominantly consisted of split liver graft, typically segment two and three. This technique allows the remaining larger portion of the liver to be used as a graft for an adult patient and has reduced both waiting list times and waiting list mortality. There have been innovations in this area however which hold a lot of promise going forward particularly in specific patient groups and circumstances.

Auxiliary liver transplantation consists of implanting a healthy liver graft either heterotopically or orthotopically while leaving the native liver in situ. Initial outcomes of this technique were poor with high rates of failure due to technical difficulties related to achieving adequate hepatic and portal

perfusion. Recent reports however have been promising and technical improvements with increased split graft and living related graft utilisation have mitigated many of these issues. There are two main areas where Auxiliary liver transplantation holds a lot of promise (115) –

As demonstrated in Appendix 1, *Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics. 2024* metabolic disease makes up 10 – 15% of the indications for liver transplantation in the Paediatric cohort. This has steadily risen over time. In many conditions transplantation occurs to provide a critical missing enzyme correcting the metabolic deficiency and in essence curing the condition. Auxiliary transplantation is this group is particularly appealing. Typically, the native liver in this group otherwise functions normally and a relatively large hepatic reserve means that only a small auxiliary graft is required to provide adequate enzyme replacement. Leaving it in situ protects the patient should the auxiliary graft fail and avoids common graft complications common in small children such as biliary anastomotic strictures. With the advent of gene therapy there is hope that in many of these conditions the underlying gene / enzyme deficits can be corrected in the future. Leaving the native liver in situ leaves the possibility of applying these future therapies potentially avoiding transplantation, its associated complications and necessary immunosuppression being a lifelong state for these patients.

Liver transplantation remains the treatment of choice for acute liver failure (ALF). Swift work up, listing and transplantation is crucial to ensure optimal outcomes. Countries such as Australia and the United Kingdom utilise priority-based listing and expediate ALF as delay in transplantation can result in patients being too unwell to undergo transplantation or succumbing prior to organ availability. The liver however is a highly regenerative organ and with time in many patients may recover. Studies have demonstrated that in select patients an auxiliary graft can be used to restore liver function and prevent brain damage and allow the native liver to regenerate preventing the need for life long immunosuppression. This holds great potential for the patient cohort described in publication 1.1, *Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med.2022* (115).

Traditional graft harvesting techniques have utilised cold storage techniques to preserve grafts by lowering their metabolic rate. Innovations in this area have yielded substantial improvements in outcomes similar to those seen with the introduction of UW (University of Wisconsin) cold storage solution. Despite this however the graft metabolic rate prior to insertion is not zero resulting in ATP depletion and ischaemic injury with longer ischaemic times resulting in poorer outcomes (116). Machine perfusion techniques aim to oxygenate the graft following harvest and during transportation. Two main forms exist. Hypothermic oxygenated machine perfusion that delivers oxygenated perfusate while lowering metabolic activity through hypothermia and normothermic regional perfusion which aims to maintain physiological conditions, provide oxygenation and nutrients. Additionally, this method of transportation reduces expression of proinflammatory cytokines, downregulation of Kupffer cells and reduced vascular resistance. Recent studies have demonstrated a modest improvement in patient survival, graft survival and biliary complications. Early allograft dysfunction appears to be significantly improved with studies suggesting an odds ratio of 0.4 comparing cold storage transplants to machine perfusion (117). This holds great promise and should be employed particularly in the repeat transplantation group described in publication 1.2, *Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021*.

2.5. Pre and Post transplant care

Wait list mortality, patient survival and graft survival post transplantation have continued to improve as demonstrated in publications appendix 1, *Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics 2024* and 3.2 *Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021*. The morbidity associated with transplantation however continues to be sobering. While patients may survive, moderate to severe intelligence quota deficits are common and may prevent patients from becoming functioning members of society (118). There is renewed interest in exploring modifiable pre- and

post-transplant factors that may improve recovery and long-term outcomes outside of graft / patient survival alone. One area of increasing recent interest is in the study of sarcopenia and frailty.

Sarcopenia is defined as a decrease in skeletal muscle mass and function and is recognised as a frequent finding in adults with cirrhosis. It is associated with increased waitlist mortality, morbidity as well as impaired posttransplant outcomes (119). The gold standard for assessment is total psoas muscle area on CT imaging however there is also research interest in measuring the muscle density to determine degree of muscle adiposity. Recent studies on sarcopenia in children have demonstrated significantly longer duration of ICU stay in children, longer hospitalization, and higher rates of infections in infants post transplantation (119,120). All these factors while clearly associated with morbidity in the immediately post operative period are also factors associated with long term complications and impairments.

The persisting question is whether sarcopenia represents a modifiable risk factor addressable with nutritional and multidisciplinary support. A pilot studies in children post-transplant utilising resistance training programs demonstrated positive results. However, adherence was an issue and further research, particularly in children pre-transplant, is required (121). Regardless this is an area of great interest going forward particularly in the context of the topics explored in publication 3.2, *Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021*.

3. Conclusion

Hepatology and transplantation are broad disciplines with Paediatric Gastroenterology. This thesis explores recent advancements and areas of need in the context of 7 main publications and 2 appendix publications.

The liver is a complex multifunctional organ with numerous roles in maintaining homeostasis. Through this complexity there is a substantial heterogeneity in paediatric liver disease. Ongoing

research and advancements provide insight into underlying pathogenic mechanisms in novel and rare conditions leading to insights into their management. This thesis explores recent advances in several rare conditions. Recently there has been a hepatitis pandemic affecting young children which appears to associated with adeno-associated virus, previously not known to cause disease in humans. Subsequent study suggests an immunological basis possibly driven by isolation practices during the SARS-CoV-2 pandemic. Due to the livers functional complexity the Paediatric Gastroenterologist must be aware of and care for children with rare genetic and metabolic liver diseases. GLIS3 mutations demonstrate a rare liver disease in which pathogenic basis of the underlying liver disease is still not fully understood and specific measures are required to achieve optimal outcomes. Liver disease in these patients appears to be a non-syndromic bile duct paucity based both on underlying histology as well as the function of the affected gene. Special care is required in these patients post transplantation due to increased risk from diabetes, infections, and chronic kidney disease. While liver malignancy remains rare in children (1.3% of malignancies in children) they demonstrate the wide diagnostic spectrum of liver diseases in children. Inflammatory Myofibroblastic tumours demonstrate the challenges of rare liver conditions with multiple therapies having been utilised to treat them with variable success. The gold standard treatment remains prompt diagnosis and where possible complete surgical excision with chemotherapy acting as an adjunctive for local spread and extensive disease burden. Outcomes are excellent where full excision is achieved which emphasises the need for prompt diagnosis to achieve this where possible. The future of this area lies within improving early detection and pick up in those at risk.

There are several areas within Paediatric Hepatology which can be identified as areas of need. Despite its prevalence and growing incidence drug induced liver injury (DILI) is an area with a paucity of evidence. Outside of very specific scenarios only supportive care is available. This is demonstrated in a case of severe DILI due to a Lamotrigine, a commonly used anti-epileptic in children, resulting in fulminant liver failure and transplantation.

Biliary disease is similarly prevalent with for the most part no treatments available to modify outcomes. Transplantation is unavoidable in progressive severe disease. We also do not know the aetiology of the most common severe biliary disease in childhood and most common cause for childhood liver transplantation, Biliary atresia. Eosinophilic cholangitis is a rarer form of biliary disease however is important to be able to recognise as one of the few forms that can be treated with medical management alone. Incorrect diagnosis, a risk given the conditions status as a diagnostic mimic, often leads to invasive interventions with increased morbidity. Population wide studies employing biobanks may shed light on this. Similarly, insights might be gained from transcriptomes of resected tissue.

Transplantation remains the final step in management of end stage liver and intestinal disease which is unable to be managed with medical therapy alone. Outcomes have drastically improved over time and with increasing patient survival more patients are requiring retransplantation for acute and chronic graft complications. Reassuringly in our cohort of children following third liver transplantation outcomes remain comparable to first and second liver transplant recipients. This study however emphasises the ongoing challenges with long term complications including mental health, intellectual impairment, and kidney disease.

Paediatric Hepatology and Transplantation are areas which are evolving rapidly. Novel diagnostics and therapies continue to advance the field. Bedside intestinal ultrasonography has been innovative for both diagnostics and monitoring in Paediatric Inflammatory bowel disease and is now utilised in clinical practice. It is particularly appealing in children as it is well tolerated and does not require general anaesthesia or ionizing radiation. The technique has promise within other areas of the Gastroenterology. Children following intestinal transplantation require even closer monitoring than those with inflammatory bowel disease due to the high risk of rejection. While endoscopically obtained biopsies for histology remain the gold standard our results demonstrate that ultrasound appears to be a similarly useful screening tool in this area utilizing similar disease cut off

measurements to those used in Paediatric inflammatory bowel disease. Other areas of future direction with promise in this area include organoid research, gene therapy techniques, fluorescence guided surgery, Auxiliary transplantation, machine perfusion of grafts and sarcopenia screening and management.

While Paediatric liver disease and transplantation management remains challenging due to diagnostic heterogeneity and the rarity of certain conditions advances continue to be made improving outcomes. Challenges remain. However ongoing research and developments presage a bright future.

APPENDIX

1. Appendix Papers and Field of Knowledge Within the Current Literature

1.1. Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades. The Journal of Pediatrics.

This paper details in retrospect the experiences of the Birmingham Children's Hospital liver unit from 1991 to 2020 with infants presenting within the first 28 days of life with neonatal liver failure (NLF, PT ≥ 20 seconds and biochemical liver injury). Patients were stratified into three 10-year block eras to analyse trends over time. It aims to evaluate trends in aetiology and outcomes of Neonatal liver failure (NLF) over 30 years retrospectively at a single institution. It links with the other papers within chapter 2, section 2 by discussing evolving diagnostics of liver disease in small children over time and highlighting areas of ongoing need (Particularly Herpes Simplex Virus and mitochondrial disease). Overall survival in NLF remains poor and this paper works to identify prognostic factors as well as highlights aetiologies with particularly poor outcomes.

Overall infective (29.5%), indeterminate (25.4%) and metabolic causes (24/6%) were the most common causes of NLF. Of the infectious aetiologies the most common cause was Herpes Simplex Virus (HSV). Infectious aetiologies generally had more severe liver injury with higher ALT and PT. Survival in the HSV group was particularly poor at 53.3%. The most common metabolic cause was Galactosaemia. Mitochondrial NLF had a particularly poor prognosis with 100% mortality. Indeterminate causes of NLF decreased over time from 42.9% in 1991-2000 to 24.6% in 2011-2020. The number of cases of gestational alloimmune liver disease (GALD) and hemophagocytic lymphohistocytosis (HLH) increased over this period. GALD was associated with a lower odds of death. The total admissions for NLF increased over time from 21 in 1991-2000 to 65 in 2011-2020. This was predominantly due to an increase in NLF secondary to underlying metabolic disorders. Overall mortality was high at 42.9% however there was improvement over time from 52.4% in 1991-2000 down to 35.4% in 2011-2020. Multiorgan failure was associated with a significant increased risk

in mortality and was seen more frequently in the infectious group. Liver transplant was performed in 18% of patients with an overall survival of 65.2% at 1 year post transplantation. All patients with HSV NLF who received a liver transplant passed away and all patients who were transplants for GALD survived.

The decrease in the number of diagnoses of NLF due to indeterminate causes over time is multifactorial with two major contributing factors. Firstly, a corresponding increase in diagnoses of GALD and HLH was also seen. The awareness, understanding and diagnostic criteria for both conditions has improved dramatically since 1990 to now, particularly in the case of GALD. Previously these cases would often be labelled as indeterminate. In the mid-2000s it was recognised that neonatal hemochromatosis, liver disease accompanied by extrahepatic siderosis, is most often a result of gestational alloimmune disease (122). Subsequent to this the only available treatments were with antioxidant treatment and liver transplantation. This realisation allowed the development of robust diagnostic guidelines and modern treatment algorithms utilizing double exchange transfusion and IvIG (122). This has revolutionised the management of this condition with contemporary studies reporting survival without liver transplant > 80% compared to 17% in historical control patients (123). Second, increased recognition of metabolic and genetic conditions and increased availability in rapid genetic testing has also greatly decreased the number of indeterminate diagnoses.

The main takeaway from this paper is the importance of rapidly establishing a diagnosis. There needs to be a low threshold to consider treatment with IVIG, antivirals, antibiotics, and galactose free formulas to treat reversible causes. However, beyond this and supportive cares, the main treatment for NLF is liver transplantation. In Australia the absolute contraindications for transplant include serious infection outside the hepatobiliary system and irreversible / progressive multiorgan disease

(32). This is emphasised in this paper with the extremely poor outcomes of infants with HSV infections and mitochondrial disease. Establishing a diagnosis for prognostication and rapid listing for transplant where appropriate is key. In the United Kingdom where this study was performed rapid whole-exome sequencing is funded and available to unwell babies admitted to NICU and PICU (124). A major challenge in an unwell neonate is obtaining the samples required for a full work up. Rapid assessment and prioritisation of testing based on likely aetiology is imperative.

While the treatment of NLF outside of transplant is mostly supportive there has been some progress in specific treatments for certain, predominantly metabolic, conditions. Tyrosinemia type 1 is an autosomal recessive condition resulting from Fumarylacetonacetate hydrolase deficiency. The lack of this enzyme prevents the breakdown of Fumarylacetoacetate which is produced due to the breakdown of dietary Phenylalanine and Tyrosine. Fumarylacetoacetate is highly reactive and its build up results in hepatocellular damage. Infants present at a few weeks of age with NLF and classically severe coagulopathy, hypoglycaemia, septicaemia, and concurrent renal tubular acidosis. Nitisinone (NTBC) was initially developed as an herbicide. It was noted however during safety testing rats exposed to NTBC developed elevated plasma tyrosine levels and ocular lesions. Further studies in 2008 demonstrated NTBC is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, an enzyme upstream to the formation of Fumarylacetoacetate. In conjunction with a low tyrosine and phenylalanine diet, it is now international consensus that NTBC be started immediately in children with Tyrosinemia type 1 on diagnosis. Early commencement has been showed to prevent the development of hepatic and renal failure, avoiding the need for transplantation (125). In recent years there has been progress in other metabolic conditions including the use of pharmaceutical chaperones (Epalrestat) for congenital disorders of glycosylation. Progress is slow however given the rarity of these conditions (126). Further developments in understanding of the underlying

aetiological basis for these conditions will continue to help the development of treatments over time.

Large retrospective cohort studies such as this one are important for two main reasons. This information provides guidance for development of treatment guidelines ongoing. This is particularly important in this area for establishing the appropriateness of listing for transplant as well as triaging of listings given the scarcity of organs. Finally, this research demonstrates areas of need to help guide future research.

Etiology, Characteristics, and Outcomes of Neonatal Liver Failure: Lessons Learned Over the Last 3 Decades

Chayarani Kelgeri, MBBS, MD, MRCPCH[1], Hari Krishnan Kanthimathinathan, MBBS, MD, MRCPCH[2], Michael Couper, MBChB, FRACP[1], Amr Alnagar, PhD, MRCS[1], Vishnu Biradar, MBBS, MD, PDCC[3], Khalid Sharif, MBBS, FRCSI, FRCS, FCPS[1], Jane Hartley, MBChB, MRCPCH, MMedSc, PhD[1], Darius Mirza, MBBS, MS, FRCS[1], and Girish L. Gupte, MBBS, MD, FRCPCH[1]

Objective To evaluate trends in etiology and outcomes of neonatal liver failure (NLF) over 30 years retrospectively at a single institution.

Study design Inclusion criteria for this retrospective cohort study were babies presenting at a chronological age of ≤28 days between 1991 and 2020 with prothrombin time ≥20 seconds and biochemical liver injury. Demographics, etiology, laboratory investigations, need for extrahepatic organ support, acute kidney injury, and intervention with liver transplant (LT) were recorded. Survival outcomes were measured as discharge from the hospital alive with native liver or LT. The study period was stratified into 3 10-year blocks. Trends were analyzed for hospital admissions, etiology, and survival outcomes.

Results One hundred twenty-six babies met the NLF criteria. Admissions to the hospital increased from 21 in 1991-2000 to 65 in 2011-2020. An increasing trend in infectious and metabolic causes, while a decreasing trend in indeterminate etiology, was noted. Survival with native liver improved from 23.8% in 1991-2000 to 55.4% in 2011-20 (P = .021), and mortality reduced from 52.4% to 35.4% during the same periods (P = .213). Twenty-three (18.2%) neonates received LT. Post-LT survival outcomes were 100% for gestational alloimmune liver disease, 66.6% in the indeterminate group, and 25% for herpes simplex virus. Specific etiologies (gestational alloimmune liver disease, OR = 0.07 [0-0.77, P = .048]), presence of acute kidney injury (OR = 6.22 [1.45, 29.38, P = .015]) and need for inotropes (OR = 6.22 [1.45, 29.38, P = .028]) influenced mortality in multivariable logistic regression analysis.

Conclusions In the last 30 years, advances in diagnosis, treatment, and increasing experience with LT have improved survival in NLF. *(J Pediatr 2024;275:114245)*.

Acute liver failure (ALF), although rare, should be suspected in all sick neonates as it can rapidly progress to multiorgan failure (MOF) and death. The Pediatric Acute Liver Failure Study Group defines ALF as liver-based coagulopathy unresponsive to vitamin K with prothrombin time (PT) ≥15 seconds(s) or international normalized ratio (INR) ≥1.5 with encephalopathy or PT ≥20 seconds or INR ≥2 without encephalopathy.[1] Given the challenges of diagnosing encephalopathy in neonates, most studies use PT ≥20 seconds or INR ≥2 as the definition of neonatal liver failure (NLF).[2,3] A prompt, comprehensive workup is undertaken to diagnose treatable etiology and prognosticate the clinical course whilst providing supportive care and timely consideration for liver transplantation (LT).

Diagnosis and evidence-based management of NLF is challenging because of its rarity, varied etiology, and phenotypic heterogeneity. Even with these limitations, the last 3 decades have seen major improvements in diagnosis and management impacting the trajectory of NLF. We report a single-center experience of NLF, analyzing the trends in etiology, outcomes, and risk factors for poor outcomes over 3 decades, from 1991 to 2020.

AFP	Alpha-fetoprotein	LT	Liver transplant
AKI	Acute kidney injury	MOF	Multiorgan failure
ALF	Acute liver failure	MRI	Magnetic resonance imaging
GALD	Gestational alloimmune liver disease	MRS	Magnetic resonance spectroscopy
		NH	Neonatal hemochromatosis
HLH	Hemophagocytic lymphocytic histiocytosis	NLF	Neonatal liver failure
		NPC	Niemann Pick C
HSV	Herpes simplex virus	PT	Prothrombin time
ICU	Intensive care unit	RRT	Renal replacement therapy
INR	International normalized ratio		

From the [1]Liver Unit Including Small Bowel Transplant, Birmingham Women's and Children's Hospital, Birmingham, United Kingdom; [2]Paediatric Intensive Care Unit, Birmingham Women's and Children's Hospital, Birmingham, United Kingdom; and [3]Paediatric Gastroenetrology and Hepatology, Jupiter Hospital, Pune, Maharashtra, India

Ethics and Data availability statement: Project approved by the hospital committee : CARMS (Clinical Audit Registration & Management system) - 00320.

https://doi.org/10.1016/j.jpeds.2024.114245

Methods

All patients with NLF admitted between January 1, 1991, and December 31, 2020, to a tertiary referral liver unit in the United Kingdom were included in this retrospective study. NLF was defined as neonates with biochemical evidence of liver injury ≤28 days of chronological age and a PT ≥20 seconds not corrected with vitamin K. Neonates with a birth gestational age <37 weeks were grouped as preterm and term babies ≤2.5 kg as low birth weight babies. All babies were investigated as per our tier 1 order set (**Supplementary Table 1**, online; available at www.jpeds.com). Results of these tests guided tier 2 investigations and tests were prioritized based on suspected etiology.[4,5] Some neonates did not complete the entire workup if a diagnosis was reached or if the baby died. Demographic details, etiology, biochemical tests (complete blood count, peak PT, alanine transaminase, serum bilirubin, alpha-fetoprotein [AFP], ferritin), genetic results, magnetic resonance imaging (MRI) of the abdomen and brain, magnetic resonance spectroscopy (MRS) of the brain, transplant interventions, microbe cultures, viral polymerase chain reaction, acute kidney injury (AKI) (defined as rise in serum creatinine above the normal range), and need for ventilatory support, inotropes, and renal replacement therapy (RRT) were recorded. The etiology of NLF was categorized into infectious, indeterminate, metabolic, GALD, primary hemophagocytic lymphocytic histiocytosis (HLH), mitochondrial, and 'other.' The criteria for diagnosis and management of each etiology are listed in **Supplementary Table 1**, online; available at www.jpeds.com. Some babies had a historical diagnosis of neonatal hemochromatosis (NH) based on hepatic siderosis and high ferritin. NH is now recognized as a phenotype resulting from different liver insults, the most common being gestational alloimmune liver disease (GALD).[6, 4] For this study, patients with a diagnosis of NH were reclassified as per the diagnostic criteria in **Supplementary Table 1**, online; available at www.jpeds.com.

Outcomes following inpatient admission for NLF were recorded as: (1) discharged alive with native liver; (2) discharged alive after LT; (3) inpatient mortality with native liver; and (4) inpatient mortality after LT.

After discharge, overall patient and graft survival (in those with LT) were recorded. All neonates with ALF received standard supportive care and etiology-specific treatment (**Supplementary Table 1**, online; available at www.jpeds.com). They were supported in the pediatric intensive care unit (ICU) if they had 1 or more failing extrahepatic organ systems (ie, need for ventilatory support, inotropes and RRT) or around the LT perioperative period.

Neonates received LT in line with category 20 (INR >4) of the National Health Services Blood and Transplant criteria for super urgent transplantation, which have remained the same over time.[7] Contraindications for LT were sepsis, diagnosis suggestive of mitochondrial, metabolic disease and HLH, and escalating extrahepatic organ support for hypoxia, hypotension, and acidosis.

To assess temporal trends in demographics, etiology, and outcomes, the study period was divided into 3 10-year blocks: 1991-2000 (era 1); 2001-2010 (era 2); and 2011-2020 (era 3). In addition, we examined risk factors for inpatient mortality.

The study follows the Strengthening the Report of Observational Studies in Epidemiology reporting guideline and is an internal audit approved by the hospital's Clinical Governance Committee.[8]

Evolution of Diagnostics and Management of NLF over the Last 3 Decades

There have been gradual advances in diagnostics, medical management, and surgical management over years that strictly do not conform to the eras used in the study period and are broadly categorized as:

Evolution of Diagnostics. Availability of polymerase chain reaction for diagnosis of viral infections (tissue and fluid cultures were used in the 90s), increasing access to MRI/MRS and genetic testing.

Evolution of Medical Management. Recognizing NH as a phenotype, use of intravenous Immunoglobulin and exchange transfusion in GALD, conservatory fluid strategies, evolution of ICU care with use of USS guided vascular access and invasive hemodynamic monitoring as standard of care, increasing use of high-volume hemofiltration techniques, safe ventilatory and neuroprotective bundles, and prevention of hospital acquired infections.

Evolution in LT Surgical Techniques in Neonates. Availability of microsurgical instruments including vascular clamps and hemostatic devices, high-resolution imaging to plan surgeries, use of intraoperative Ultrasound and dopplers, use of recipient size appropriate reduced and mono segment liver grafts.

Statistical Analysis

We used Microsoft Excel, SPSS version 28.0 (IBM Corp), and R (R Project for statistical computing) for statistical analysis. Data are presented as median (IQR) or numbers (percentage). The categorical variables were compared using a χ^2 or Fisher exact test. A P value < .05 was considered significant. The Kruskal-Wallis test for independent samples was used for continuous variables as the data were skewed and did not support the normal distribution assumption. Kaplan-Meier survival curves were used to study the survival of the patients over time, for different time periods and etiologies. Log-rank tests were used to compare the survival functions between the time periods and different etiologies. Conditional survival curves were computed using the Kaplan-Meier method for the survivors beyond 1 year. In addition, univariable and multivariable logistic regression models were used to describe the associations between in-hospital mortality and several demographic, clinical predictors, and etiology of NLF.

Results

Of the 126 neonates with a diagnosis of NLF admitted during the study period, 98 (78%) were Caucasians, and 50% were female. The median weight across all etiologies was 2.9 Kg (2.5-3.4), and 27 (21.4%) neonates were preterm. Overall infectious, indeterminate, and metabolic were the most common causes of NLF at 29.5% (n = 37), 25.4% (n = 32), and 24.6% (n = 31), respectively.

Temporal Trends in Demographics and Etiology

The demographic, etiologic, and outcome data are shown in Table I and Figures 1 and 2. The trend in sex distribution, weight, and ethnic background of the study cohort did not change over time. The total admissions increased from 21 in 1991-2000 to 65 in 2011-20, primarily due to an increase in NLF secondary to an underlying metabolic disorder (from 1 to 15) and herpes simplex virus (HSV)-NLF (from 2 to 15) during the same period. A decline in indeterminate NLF was noted, from 42.9% in 1991-00 to 24.6% in 2011-20. The number of cases of GALD, HLH, and others increased over time, though the absolute numbers remain small.

HSV was the most common cause in the infectious group, with 24 neonates having HSV-NLF, 20 due to HSV-1 and 4 due to HSV-2. Eight babies were diagnosed with enterovirus infection, and 1 baby had disseminated cytomegalovirus as the cause of NLF. Four babies had NLF due to bacterial infections. The metabolic group (n = 31) included 23 neonates with galactosemia, 3 with tyrosinemia, 2 with ornithine transcarbamylase deficiency, and 1 each with Niemann Pick C (NPC), Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency and neonatal Gaucher disease. Nine neonates received a diagnosis of GALD. Lip biopsy for iron staining in the salivary gland was positive in 4, negative in 3, insufficient in 1, and missing result in 1. MRI abdomen was performed on 7, of which 6 demonstrated pancreatic iron deposition. Two neonates were positive for extrahepatic iron both in the lip biopsy and on MRI abdomen. One baby with a nondiagnostic lip biopsy did not have evidence of extrahepatic iron deposition on MRI abdomen but received a diagnosis of GALD because of a previously affected sibling with GALD. Seven neonates had mitochondrial NLF, 1 with MPV 17, 2 with deoxyguanosine kinase deficiency, 1 with mitochondrial depletion, 1 had MEGDEL: 3-methylglutaconic aciduria, deafness (D), encephalopathy (E), and Leigh-like disease (L) caused by serine active site containing 1 mutation, 1 with complex I and IV deficiency, 1 with MRI and MRS abnormalities suggestive of mitochondrial disease. Four neonates had primary HLH. The 'others' category included 3 neonates with hemangioma and 1 neonate each with hemangiopericytoma, systemic lupus erythematosus, and hypoxic-ischemic encephalopathy. Of the 3 babies with hemangioma, 2 had NLF following therapeutic embolization. Genetic testing facilitated a diagnosis in 12 babies.

Laboratory Parameters

Laboratory parameters in each category were reviewed (Supplementary Table 2, online; available at www.jpeds.com). High PT with near normal transaminases in GALD, high PT with high transaminases in infection, modest elevation of PT and transaminases in metabolic disease and HLH were noted. Ferritin was highest in HLH and infection. Eighteen babies with NLF had a bone marrow examination, of which 6 had hemophagocytosis (2 with HSV, 1 with mitochondrial, 1 with lupus, and 2 with primary HLH). AFP was lower in babies with HLH (median <212 ng/mL), and

Table I. Demography, etiology, and outcome trend over the study period 1991-2020

Variable	Overall	1991-00	2001-10	2011-20	*P* value
n	126	21	40	65	
Sex = male (%)	63 (50.0)	11 (52.4)	23 (57.5)	29 (44.6)	.427
Race (%)					.850
Other	2 (1.6)	0 (0.0)	1 (2.5)	1 (1.5)	
Asian	26 (20.6)	3 (14.3)	8 (20.0)	15 (23.1)	
Caucasian	98 (77.8)	18 (85.7)	31 (77.5)	49 (75.4)	
Gestation = preterm (%)	27 (21.4)	7 (33.3)	7 (17.5)	13 (20.0)	.331
Weight (median [IQR]) kg	2.9 [2.5, 3.4]	2.9 [2.6,3.2]	2.9 [2.5, 3.5]	2.9 [2.5,3.4]	.932
Etiology (%)					.065
Infection HSV	24 (19.0)	2 (9.5)	7 (17.5)	15 (23.1)	
Infection non-HSV	13 (10.3)	5 (23.8)	3 (7.5)	5 (7.7)	
GALD	9 (7.1)	1 (4.8)	4 (10.0)	4 (6.2)	
Metabolic	31 (24.6)	1 (4.8)	14 (35.0)	16 (24.6)	
Indeterminate	32 (25.4)	9 (42.9)	7 (17.5)	16 (24.6)	
HLH	4 (3.2)	0 (0.0)	0 (0.0)	4 (6.2)	
Others/mitochondrial	13 (10.3)	3 (14.3)	5 (12.5)	5 (7.7)	
Transplant listing (%)					**.041**
Not listed	97 (77.0)	14 (66.7)	26 (65.0)	57 (87.7)	
Listed for transplant	29 (23.0)	7 (33.3)	14 (35)	8 (12.3)	
Transplanted	23 (18.3)	6 (28.6)	10 (25.0)	7 (10.8)	
Outcome = died (%) 0.213					
Died after transplant	8 (6.3)	1 (4.8)	6 (15)	1 (1.5)	
Died with native liver	46 (36.5)	10 (47.6)	14 (35.0)	22 (33.8)	

kg, Kilogram; *n*, Number.
Bold indicates $P < .05$ statistically significant.

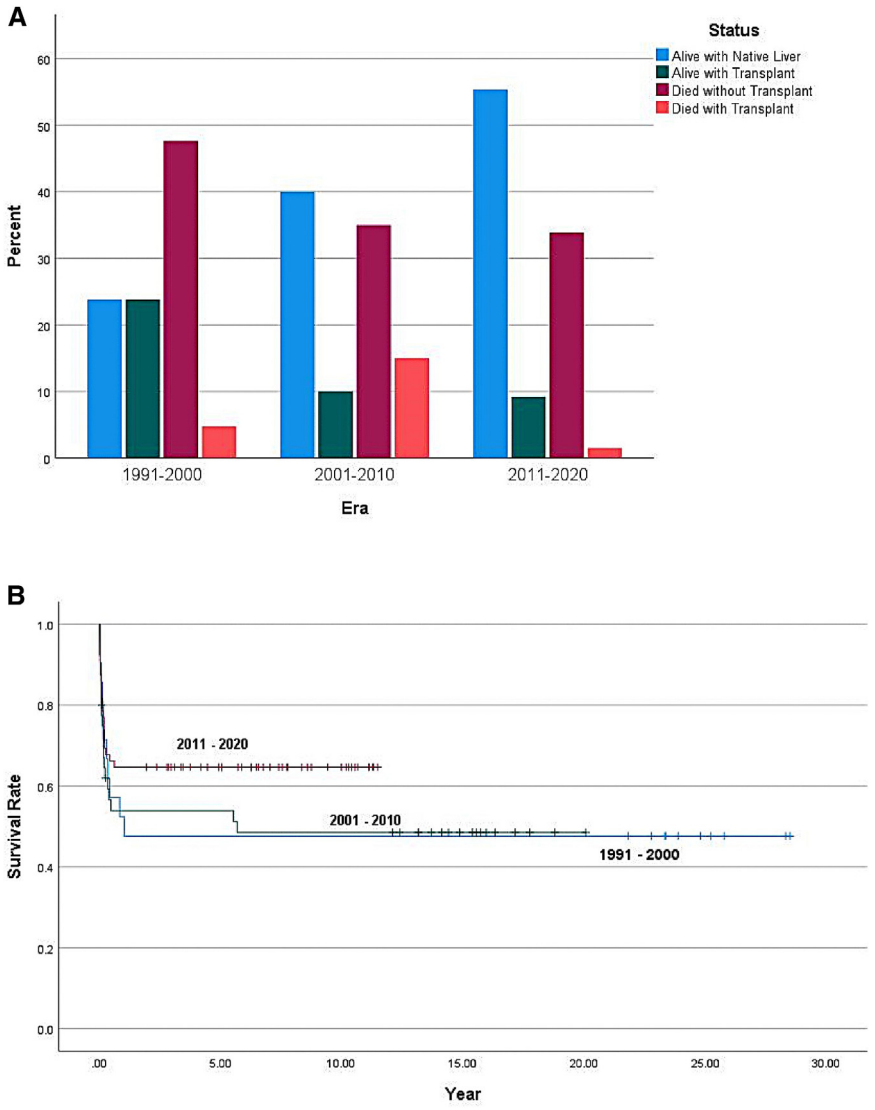


Figure 1. Outcome of the patients in different eras. **A,** Survival and death with native liver or post-transplant. **B,** Kaplan-Meier survival curve of the patients in different eras for all patients.

infections (median <5000 ng/mL) compared with other etiologies (median >10 000 ng/mL). AFP was also noted to be lower in babies who died with native liver or received LT (median 19 000 ng/mL) compared with those who survived with native liver (median 24 061 ng/mL), although this was not statistically significant ($P = .557$).

Temporal Trends in Outcomes and Poor Prognostic Factors

The overall inpatient mortality over the study period was 42.9% (n = 54), with an improving trend of reduction in mortality from 52.4% in 1991-00 to 35.4% in 2011-20 (Figure 1), though this is not statistically significant ($P = .213$). Of the 4 neonates with primary HLH, 1 survived following a stem cell transplant. Survival in HSV-NLF remained poor, ranging from 14.2% to 53.3% over the study period. Six of 7 babies with HSV-NLF died in 2001-2010, 3 with native liver and 3 after LT (1 urvived with native liver), contributing to the higher inpatient mortality for that era. Overall, of the 24 babies with HSV, 9 survived, 1 following LT and 8 with native liver. Deaths in the metabolic group included 3 with galactosemia and fulminant sepsis, 2 with ornithine transcarbamylase, and 1 with Gaucher disease (Figure 2). In the 'Other' category, 2 babies who developed NLF following embolization of hemangioma and 1 with hemangiopericytoma died.

Higher admission weights appeared to be associated with reducing mortality in univariable analysis (OR = 0.56, $P = .057$), though the association disappeared when adjusted for other variables in the multivariable analysis (Table II). When using HSV etiology as the reference group in the multivariable analysis, a diagnosis of GALD was significantly associated with lower odds of death with an

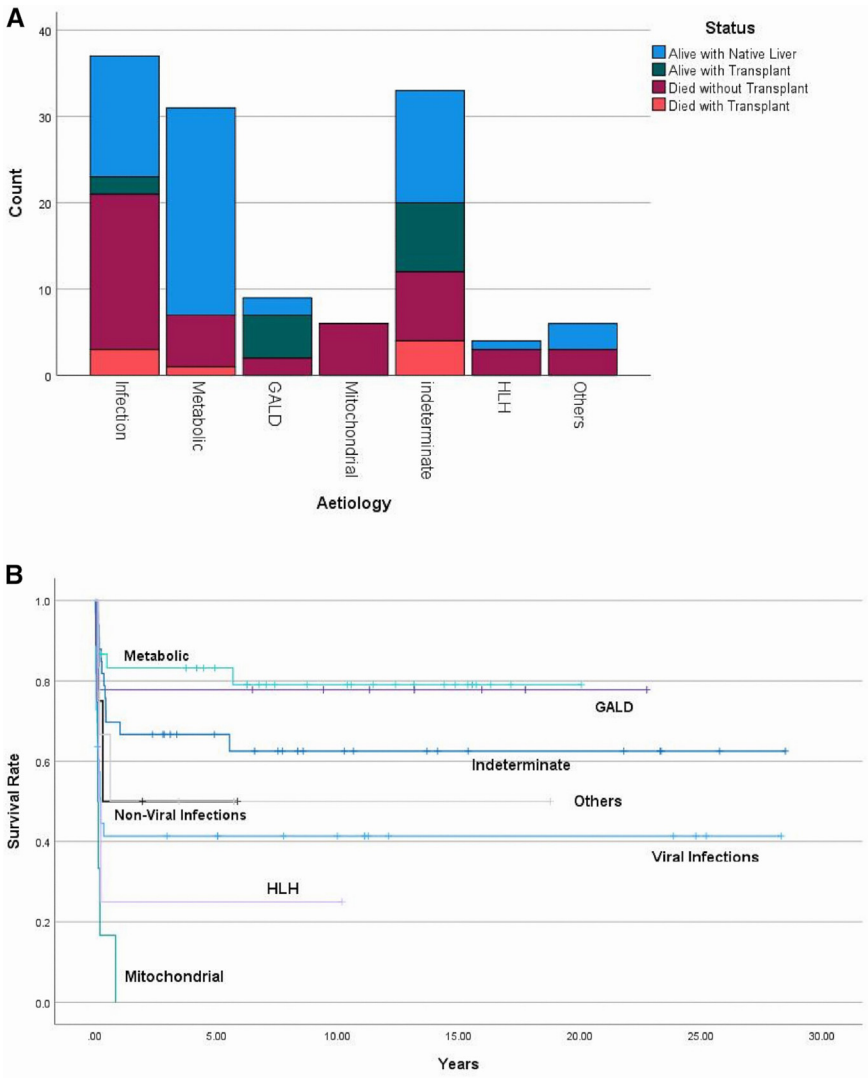


Figure 2. Outcome of the patients with different etiology. **A,** Survival with native liver or liver transplant; **B,** Kaplan-Meier survival curve for different etiology. *GALD*, gestational alloimmune liver disease; *HLH*, hemophagocytic lymphocytic histiocytosis.

OR of 0.07 (0.0-0.77, P = .048). No other variables were significantly associated with mortality when considered in the univariable or multivariable model.

About half the babies (n = 70, 55.5%) had at least 1 failing extrahepatic organ. A third (n = 38, 30.2%) of them had respiratory failure, ionotropic requirement and AKI, and were associated with significant mortality (P values < .001). The mortality associations persisted with a 6-fold increase in the OR of death in a multivariable analysis for AKI and cardiovascular support but not for respiratory support alone. Seventy babies required ICU admission (56% of all NLFs) during the study period. The proportion of babies admitted to ICU remained consistent [12/21 (57%), 22/40 (55%), and 36/65 (55%), respectively, during the 3-eras]. Of the 70 babies needing extra hepatic organ support, 62 needed invasive ventilation (89%), 45 required inotropic support (64%), and 23 babies required RRT (33%). The overall mean (SD) length of stay in ICU during the study period was 12.3 days (13.5), and this remained consistent during the eras (mean: 11.3, 12.1, 12.6 days respectively).

Survival with native liver increased from 23.8% in 1991-2000 to 55.4% in 2011-2020 (P = .021), with fewer NLF listed for LT (33% in 1991-00 to 12% in 2011-20; P = .041).

Liver Transplant

A total of 29 babies were listed for LT, of which 23 (18%) received a LT (**Figure 1**, A). One baby was delisted following resolution of liver failure. The remaining 5 deteriorated while being on the waiting list and died. Of the babies receiving LT, the median time on the waiting list was 8 days (range 1-28), the median age at transplant was 20 days (range 11-164), and the recipient median weight was 3.35 Kg (range 2-4.9). The median donor age and weight was 8 years (3 months-53 years) and 25 Kg (6-65) respectively. All liver grafts were technical variant grafts from deceased donors.

Table II. Logistic regression results showing associations with mortality

Variable	Univariable analysis		Multivariable analysis	
	OR [95% CI]	*P* value	OR [95% CI]	*P* value
Etiology				
Reference = infection (HSV)	-	-	-	-
Infection (non-HSV)	0.51 [0.13, 2.01]	.341	0.24 [0.03, 1.97]	.190
GALD	0.17 [0.02, 0.89]	.052	0.07 [0.00, 0.77]	**.048**
Metabolic	**0.18 [0.05, 0.55]**	**.004**	0.86 [0.13, 6.06]	.873
Indeterminate	**0.31 [0.10, 0.93]**	**.040**	1.01 [0.16, 7.45]	.988
HLH	1.80 [0.20, 39.50]	.633	0.35 [0.02, 11.94]	.506
Others/mitochondrial	2.00 [0.46, 10.71]	.375	8.75 [0.96, 103.37]	.065
Gestation = preterm (vs term)	1.92 [0.82, 4.62]	.136	0.72 [0.14, 3.64]	.694
Weight (kg)	0.56 [0.30, 1.00]	.057	0.32 [0.09, 1.01]	.064
Ventilated = yes (vs no)	**10.90 [4.83, 26.40]**	**<.001**	3.17 [0.71, 14.03]	.124
AKI = yes (vs no)	**13.07 [5.73, 31.89]**	**<.001**	**6.22 [1.45, 29.38]**	**.015**
Inotropes = yes (vs No)	**11.09 [4.86, 27.14]**	**<.001**	**6.19 [1.27, 34.06]**	**.028**
Transplanted = yes (vs no)	0.66 [0.25, 1.66]	.389	0.64 [0.13, 3.14]	.576

kg, Kilogram.
Bold indicates P < .05 statistically significant.

Inpatient survival after LT in 1991-2000, 2001-2010, and 2011-2020 was 83.3%, 40% and 85.7%, respectively. A poor inpatient post-LT survival of 40% (n = 4, *P* = .023) recorded in 2001-2010 was primarily a result of high mortality from HSV-NLF. Three of 7 babies with HSV-NLF in this period were transplanted, and all died. Overall survival was higher in the transplant group; though statistically not significant (*P* = .368) (**Figure 3**, A). Of the babies receiving LT (n = 23), all with GALD (n = 5100%), 8 in the indeterminate group (n = 12, 66%) and 1 each of HSV (n = 4,25%), *Enterovirus* (n = 1100%), NPC (n = 1100%) were discharged home after a median hospital stay of 40 days (range 25-59) after LT. Two patients were retransplanted, one because of nonfunctioning liver graft a day after the first LT and the second patient, 15 years later because of chronic rejection leading to graft loss. Overall patient survival at 1-year and 5-year postdischarge was 65.2%, and 60.8% respectively.

Mortality Postdischarge

There were 8 deaths after discharge from the hospital. Two babies with NLF were discharged with a palliative care plan and died after 5 and 10 days, respectively. They were included in the inpatient mortality for analysis. One baby in the early 90s from the indeterminate group progressed to chronic liver disease with portal hypertension and died postvariceal bleed at 8 months of age.

Out of the remaining 5, 2 died due to complications of their underlying disease unrelated to the liver failure and 1 due to disseminated cytomegalovirus. Two deaths were recorded in the post-LT cohort, the neonate with NPC died 5 years post-LT from progressive neurological disease and 1 died at 23 years of age from an unknown cause. The conditional survival beyond 1 year is constant for all etiologies, as shown in **Figure 3**, B.

Discussion

We report a retrospective single-center study of a large cohort of 126 neonates with ALF, a rare condition, admitted to a tertiary pediatric liver unit in the United Kingdom from 1991 to 2020. Infection, metabolic, and indeterminate were the most common causes of NLF across the study period. The presence of extra hepatic organ failure and underlying etiology (mitochondrial, HLH, and HSV-NLF) were significantly associated with increased mortality. Hospital admissions for NLF and survival, particularly with native liver, have increased over time. Survival post-LT is favorable for GALD and the indeterminate group but remains poor in HSV-related NLF.

NLF has been reported in retrospective small case series from various centers, some including infants up to 12 months old, and more recently as a retrospective multicenter study from the USA.[2,3,8-12] Evolving knowledge of conditions like GALD and NH, heterogeneity in the data, and differences in categorization (with some including metabolic and mitochondrial as a genetic group) complicate outcome comparisons. Nevertheless, we have attempted to compare similar cohorts and make several important observations.

Referrals of babies with NLF have consistently increased over the years, possibly due to heightened awareness and the availability of LT. In most case series, infections, ischemic insults, metabolic causes, and GALD (or NH in historical studies) are most typical followed by mitochondrial NLF and Primary HLH.[2,3,8-12] They are reported with varying frequency, probably reflecting referral patterns rather than the true epidemiology. Other causes of NLF reported are systemic lupus erythematosus, bile acid synthetic defects, hypopituitarism, trisomy 21 associated transient-abnormal myelopoiesis, and maternal drugs.[2,4,9,13] Metabolic causes are reported less frequent in a study from US, as some are diagnosed on newborn screening.[2]

Although many studies have proposed a higher PT for diagnosis of NLF, it is recommended that babies with PT >15 seconds and evidence of biochemical liver injury should be investigated promptly and monitored closely. Six babies in our cohort had PT between 17 and 19 s at presentation but eventually worsened, and 5 of these succumbed.

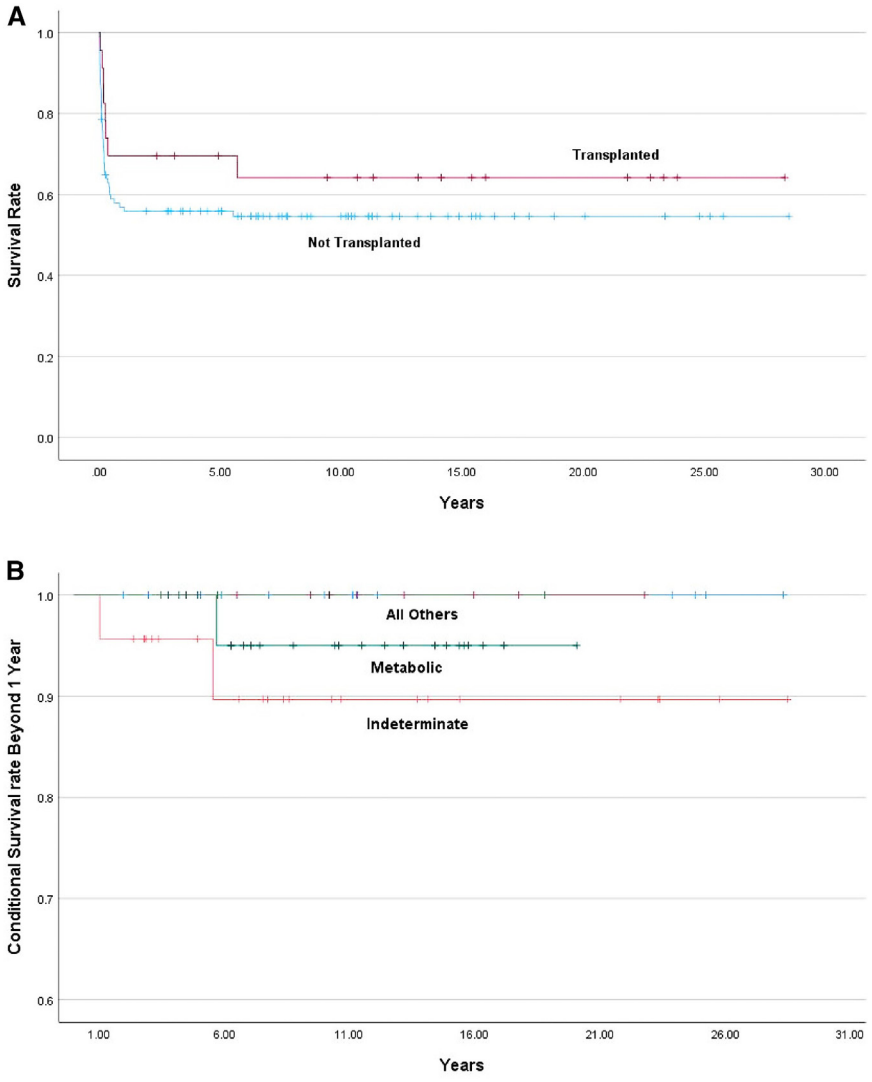


Figure 3. **A,** Kaplan-Meier survival curves of the patient survival with native liver and with liver transplant, **B,** Conditional Kaplan-Meier survival curves beyond 1 year of survival for different etiology.

Our study reinforces the importance of etiology for prognostication in NLF. However, investigating NLF is challenging due to limited blood volume and rapid deterioration. The initial pattern of laboratory parameters for different etiologies in our study is similar to what has been reported previously.[5] Prioritizing investigations guided by liver biochemistry helps in expediting a diagnosis.

The exact frequency of GALD is challenging to interpret as historical studies, including 1 from our center, have used hepatic siderosis for diagnosis and categorized them as NH.[12,14] Positive C5b-9 staining in the liver has been used to diagnose GALD, but this is not available in the United Kingdom and is not specific to GALD.[15]

HSV-NLF is the most common cause of viral infection in most series.[2,3,5,12] Other studies, like ours, have reported secondary HLH in HSV-NLF.[16,17] An important implication of this finding is to investigate the use of concurrent immunotherapy with steroids and/or chemotherapy, as antivirals alone may be insufficient to overcome the cytokine storm and control hyperinflammation.[17-19] The fulminant necrosis and limited regeneration of the hepatocytes is likely reflected by the low AFP levels seen in the HSV group.

Variations in age inclusion and etiology among different series influence the reported incidence of indeterminate diagnosis. Centers in the United Kingdom and US report 6%-38% of babies with indeterminate diagnosis compared with 26.2% in our cohort.[2,3,5,9] The higher incidence reported in our study is because of applying strict age and diagnostic criteria. Further, our cohort does not include NLF due to ischemic encephalopathy and bacterial infection as they are managed in neonatal units and not transferred to the liver center. Studies that include NLF due to these etiologies will have a diluted incidence of indeterminate diagnosis. Whole genome or exome sequence testing in the United Kingdom, currently is not freely available and needs to be preapproved

by the geneticist. Easy access to genetic testing will further reduce the incidence of indeterminate diagnosis.

Mortality in NLF is high, reported between 53% and 67% with risk factors being the underlying etiology and presence of extrahepatic organ failure.[3,12] Two studies report mortality of 50% in NLF who require extrahepatic organ support at 30 days of life and 64% in babies <90 days with ALF and MOF.[2,5] The US multicentric study reports respiratory failure needing ventilatory support to be the strongest correlator of poor outcomes in contrast to our cohort, which found AKI to be associated with poor outcomes.[3] The highest mortality (77.7%, n = 18) in our cohort was recorded with all 3 extrahepatic organ failures, and this was most common in the infection group (n = 10, 55.5%).

LT in NLF accounts for <2% of all pediatric LT and post-LT outcomes in neonates have been reported to be similar to those in older infants.[3,20,21] Our data are comparable to other series reporting 5%-22% of NLF needing LT, the most common indication being indeterminate, NH, and GALD.[2,3,5,11,20,21] LT, while successful in some HSV-NLFs, including 1 from our center, generally has poor survival.[16,22-24] Babies with HSV infection can have prolonged viremia and often have MOF.[25] The high mortality post-LT noted in the HSV-NLF cohort led us to review our practice and currently LT is considered in a select few whose ALF does not improve with supportive care, have received high dose Acyclovir (60 mg/kg/day, dose modified in presence of AKI) for at least 5 days and are not in MOF. Lumbar puncture cannot be performed in view of coagulopathy and thrombocytopenia, so neurological examination, brain imaging, and electroencephalogram are used as surrogate tests for neurological involvement. This approach is not evidence based and needs multicenter studies with larger samples to guide LT candidacy in HSV-NLF. Similar to our cohort, HSV-NLF at other centers too were less likely to receive LT.[3] The overall post-LT outcomes in our cohort are similar to those reported in other series.[3,20,21]

The survival in our series has improved (57.1%, n = 72 overall and 61.1%, n = 44 in the last decade) and seems to be better than reported in other studies.[2,3]

Limitations of this study include its retrospective nature and missing historical data. The diagnostic criteria for neonatal AKI have changed over time, and hence, using only serum creatinine above the normal range may possibly underestimate AKI in our cohort.

In conclusion, the survival of babies with NLF has improved over the past 30 years. Developments in diagnostic availability and techniques, and evolution of multiorgan supportive care for neonates has contributed to improving outcomes. LT remains an effective lifesaving option in a select group of patients with NLF. ■

CRediT authorship contribution statement

Chayarani Kelgeri: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hari Krishnan Kanthimathinathan:** Software, Formal analysis. **Michael Couper:** Writing – review & editing. **Amr Alnagar:** Writing – review & editing, Data curation. **Vishnu Biradar:** Data curation. **Khalid Sharif:** Writing – review & editing. **Jane Hartley:** Writing – review & editing, Investigation. **Darius Mirza:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Girish L. Gupte:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Submitted for publication Jan 31, 2024; last revision received Aug 7, 2024; accepted Aug 12, 2024.

Reprint requests: Dr Chayarani Kelgeri, MBBS, MD, MRCPCH, Liver Unit, Consultant Paediatric Hepatologist, Birmingham Women's, and Children's NHS Foundation Trust, Steel House Lane, Birmingham B4 6NH, UK. E-mail: chayarani.kelgeri@nhs.net

References

1. Squires RH Jr, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr 2006;148:652-8.
2. Borovsky K, Banc-Husu AM, Saul SA, Neighbors K, Kelly S, Alonso EM, et al. Applying an age-specific definition to better characterize etiologies and outcomes in neonatal acute liver failure. J Pediatr Gastroenterol Nutr 2021;73:80-5.
3. Antala S, Whitehead B, Godown J, Hall M, Banc-Husu A, Alonso EM, et al. Neonates with acute liver failure have higher overall mortality but similar post transplant outcomes as older infants. Liver Transpl 2023;29:5-14.
4. Taylor SA, Whitington PF. Neonatal acute liver failure. Liver Transpl 2016;22:677-85.
5. Sundaram SS, Alonso EM, Narkewicz MR, Zhang S, Squires RH, Pediatric Acute Liver Failure Study Group. Characterization and outcomes of young infants with acute liver failure. J Pediatr 2011;159:813-8.
6. Shneider BL. Neonatal liver failure. Curr Opin Pediatr 1996;8:495-501.
7. NHS Blood & Transplant. POL195/18: liver transplantation: selection criteria and recipient registration. 2024. Accessed July 1, 2024. nhsbtdbe.blob.core.windows.net
8. STROBE checklist: cohort studies. EQUATOR Network. 2015. Accessed July 1, 2024. https://www.equator-network.org/wp-content/uploads/2015/10/STROBE_checklist_v4_cohort.pdf
9. Bitar R, Thwaites R, Davison S, Rajwal S, McClean P. Liver failure in early infancy: aetiology, presentation, and outcome. J Pediatr Gastroenterol Nutr 2017;64:70-5.
10. Durand P, Debray D, Mandel R, Baujard C, Branchereau S, Gauthier F, et al. Acute liver failure in infancy: a 14-year experience of a pediatric liver transplantation center. J Pediatr 2001;139:871-6.
11. Di Giorgio A, Sonzogni A, Piccichè A, Alessio G, Bonanomi E, Colledan M, et al. Successful management of acute liver failure in Italian children: a 16-year experience at a referral centre for paediatric liver transplantation. Dig Liver Dis 2017;49:1139-45.
12. Shanmugam NP, Bansal S, Greenough A, Verma A, Dhawan A. Neonatal liver failure: aetiologies and management–state of the art. Eur J Pediatr 2011;170:573-81.
13. AW MM, Dhawan A, Baker AJ, Mieli-Vergani G. Neonatal paracetamol poisoning. Arch Dis Child Fetal Neonatal Ed 1999;81:78.
14. Flynn DM, Mohan N, McKiernan P, Beath S, Buckels J, Mayer D, et al. Progress in treatment and outcome for children with neonatal haemochromatosis. Arch Dis Child Fetal Neonatal Ed 2003;88:124-7.

15. Dubruc E, Nadaud B, Ruchelli E, Heissat S, Baruteau J, Broue P, et al. Relevance of C5b9 immunostaining in the diagnosis of neonatal hemochromatosis. Pediatr Res 2017;81:712-21.
16. Egawa H, Inomata Y, Nakayama S, Matsui A, Yamabe H, Uemoto S, et al. Fulminant hepatic failure secondary to herpes simplex virus infection in a neonate: a case report of successful treatment with liver transplantation and perioperative acyclovir. Liver Transpl Surg 1998;4:513-6.
17. Suzuki N, Morimoto A, Ohga S, Kudo K, Ishida Y, Ishii E, et al. Characteristics of hemophagocytic lymphohistiocytosis in neonates: a nationwide survey in Japan. J Pediatr 2009;155:235-8.
18. Kawada J, Kimura H, Ito Y, Ando Y, Tanaka-Kitajima N, Hayakawa M, et al. Evaluation of systemic inflammatory responses in neonates with herpes simplex virus infection. J Infect Dis 2004;190:494-8.
19. Gantt S, Muller WJ. The immunologic basis for severe neonatal herpes disease and potential strategies for therapeutic intervention. Clin Dev Immunol 2013;2013:369172.
20. Sundaram SS, Alonso EM, Whitington PF. Liver transplantation in neonates. Liver Transpl 2003;9:783-8.
21. Grabhorn E, Richter A, Fischer L, Ganschow R. Emergency liver transplantation in neonates with acute liver failure: long-term follow-up. Transplantation 2008;15:932-6.
22. Lee WS, Kelly DA, Tanner MS, Ramani P, de Ville de Goyet J, McKiernan PJ. Neonatal liver transplantation for fulminant hepatitis caused by herpes simplex virus type 2. J Pediatr Gastroenterol Nutr 2002;35:220-3.
23. Twagira M, Hadzic N, Smith M, Ramaswamy M, Verma A, Dhawan A, et al. Disseminated neonatal herpes simplex virus (HSV) type 2 infection diagnosed by HSV DNA detection in blood and successfully managed by liver transplantation. Eur J Pediatr 2004;163:166-9.
24. Ichai P, Roque Afonso AM, Sebagh M, Gonzalez ME, Codés L, Azoulay D, et al. Herpes simplex virus-associated acute liver failure: a difficult diagnosis with a poor prognosis. Liver Transpl 2005;11:1550-5.
25. Melvin AJ, Mohan KM, Schiffer JT, Drolette LM, Magaret A, Corey L, et al. Plasma and cerebrospinal fluid herpes simplex virus levels at diagnosis and outcome of neonatal infection. J Pediatr 2015;166:827-33.

1.2. South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1-associated hereditary pancreatitis. Med J Aust.

This paper is a retrospective study and perspective describing the outcomes of five patients with PRSS mutations who underwent total pancreatectomy and islet auto transplantation (TP-IAT) at the Women's and Children's Hospital from the inception of the program. It aims to explore the South Australian experience with this procedure – an innovation on previous chronic pancreatitis surgery, as well as explore factors associated with outcome. It links in with other papers in this thesis discussing an area of advancement in the management of this patient group. Similar this is an area of need particularly within South Australia, where this thesis's author practices, which has a much higher incidence of hereditary pancreatitis resulting in significant morbidity.

Patients were predominantly female and of Aboriginal and Torres Strait Islander background. PRSS mutations are 70-fold more common in these ethnic groups explaining the higher numbers. The procedure was performed in a wide range of ages (range 6.9 to 17.8 years, median 16.8 years). High yields of islet cells per kilogram of body weight (> 2500 IEQ/kg) have previously been associated with better outcomes. This was achieved in four patients, with at the time of reporting, two patients achieving long term insulin independence and a third with negligible requirements. The fourth developed an insulin requirement following development of glutamic acid decarboxylase islet antibodies 20 months following the procedure. The fifth patient with low yield developed a long-term insulin dependence but good glycaemic control suggests some engraftment. All five patients had no ongoing opiate requirement and were able to resume normal activities of daily living (Hospital admissions for pancreatitis prior to procedure median 13.5, range 11.5 – 20.0).

TP-IAT was pioneered at the University of Minnesota with the first human procedure performed there in 1977. The University of Minnesota criteria for patient selection was subsequently developed and remains in widespread use today (Table 1 – University of Minnesota Criteria for total

pancreatectomy and islet auto transplantation, page 209 (46)). Consequently, most cases have been performed in Minnesota allowing for the largest single centre data set to evaluate outcomes. The largest study from 1977 to 2011 from Minnesota demonstrated an insulin independence rate of 28% and 30% at 1 and 3 years respectively. Narcotic independence at 2 years was 59%. Average islet yield was 3050 IEQ/kg (46). Within the limits of a small patient cohort, the experience in Adelaide has been much more optimistic for long term insulin and narcotic independence. This is despite islet cell harvesting occurring interstate following initial total pancreatectomy (In the morning) resulting in some delays in re-engraftment (Evening following initial total pancreatectomy, range 11 – 13 hours later).

There are several factors which may contribute to this discrepancy. The mean age of patients undergoing TP-IAT at Minnesota was 35.3 years. Meta-analysis has demonstrated that children do have higher rates of insulin-independence post procedure (47.7% vs 31.8%) however this difference on its own is still modest (127). Additionally, it is recognised that an early operation leads to a higher islet yield and improved outcomes. With an average symptom duration of 9.2 years in the Minnesota cohort this may further amplify the discrepancies seen between Adult and Paediatric patients. Furthermore 21% of the Minnesota cohort had undergone prior surgery which has been associated with poorer islet yield, increased fibrosis, and pain ((46), (127)). Two substantial differences in the post operative protocol used in Adelaide is a lack of enteral tube feeds and the use of sophisticated pump technology during the immediate post operative period. With most of the adult literature focusing on narcotic independence as the main outcome measure studies on whether early tight glycaemic control influences long term insulin independence do not exist. It has been noted that females, lower body weight and lower mean insulin requirements in the first 24 hours post operatively are associated with insulin independence (128). It is conceivable that tight early control allows a better transition post operatively and long-term engrafted islet function however further study with larger cohorts is required.

Two developments may influence the future of auto islet transplantation in Australia. First, as more centres begin to perform the procedure in Australia in children and adults' questions arise as to what constitutes an adequate case load for a centre. This is critical both to maintain expertise and to justify the expensive facilities and experienced staff that are required. For example, would Australia be best served by no more than two to three centres, as for the original islet transplant program in Sydney, Melbourne, and Adelaide? A similar set up is currently seen with both Liver and Intestinal transplantation programs in Melbourne, Sydney and Brisbane and Melbourne alone for the former and later respectively. Second, stem cell therapies are finding some early success in trials in type 1 diabetes even with short term insulin independence reported in some participants. Clinically approved stem cell-derived insulin-producing cells licensed for use in trials are multipotent pancreatic progenitors implanted subcutaneously before full maturation with different encapsulation models (129). This ground breaking area of research may increasingly over take islet transplantation in type 1 diabetes management and so reduce the pool of islet biologists available for the harvesting of islets in islet auto-transplants.

TP-IAT is a promising technique particularly in children. Early experience in Australia has been particularly positive likely due to tight glycaemic control in the post operative period. The technique has proven to be the standard for chronic pancreatitis supplanting traditional procedures. Ongoing studies to determine factors resulting in improved rates of insulin and narcotic independence will lead to ongoing improvements in outcomes with the current literature supporting early consideration for the procedure to maximize islet cell yield. Within Australasia consideration of national referral centres to maintain expertise is needed. Stem cell therapies while not immediately applicable to chronic pancreatitis and narcotic dependence may have a future role in type 1 diabetes management and insulin independence.

South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1 associated hereditary pancreatitis

McCole JA(1), Couper MR(1), Moore DJ(1,2), Khurana S (2,3), Chen JW (4,5), Drogemuller CJ (2,6), Radford T (6), Couper JJ (2,7,8), Wilks M (9,10,11,12), Kay TW (13,14), Loudovaris T (13), Coates PA(2, 6), Couper RTL (1,2).

Affiliations

1. Department of Gastroenterology, Women's & Children's Hospital, South Australia
2. School of Medicine, University of Adelaide, Adelaide, South Australia
3. Department of Paediatric Surgery and Urology, Women's & Children's Hospital, South Australia
4. South Australian Liver Transplant Unit and Hepatopancreatobiliary Unit, Flinders Medical Centre, South Australia
5. School of Medicine, Flinders Medical Centre, South Australia
6. Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, South Australia
7. Department of Endocrinology and Diabetes, Women's & Children's Hospital, South Australia
8. Robinson Research Institute, South Australia
9. Department of Radiology, Women's & Children's Hospital, South Australia
10. Department of Radiology, Royal Adelaide Hospital, South Australia
11. Radiology SA, Adelaide, South Australia
12. Cook Medical Australia
13. Immunology and Diabetes, St Vincent's Institute, Melbourne, Australia
14. School of Medicine, University of Melbourne, Australia

Abstract

Objective: Hereditary gene mutations of PRSS1 cationic trypsinogen gene are a common cause for acute recurrent or chronic pancreatitis, and are associated with the evolution of endocrine and exocrine dysfunction, and risk of pancreatic cancer long-term. Total Pancreatectomy and islet autotransplantation (TP-IAT) is an established treatment for children with severe hereditary pancreatitis related morbidity.

Design, setting and participants: Single arm observation study of 5 children with a PRSS1 mutation and chronic pancreatitis who underwent TP-IAT at our institution between 2015 and 2020, the largest paediatric cohort in Australia. All patients fulfilled published University of Minnesota criteria and recommendations from PancreasFest for TP-IAT.

Main outcome measures: islet yield, long term independence from exogenous insulin and opioid analgesia.

Results: Four of five children had good islet yield with high detectable c-peptide levels post-operatively. Two children remain insulin-independent long term, one child has declining requirements in the early post-operative period, and a further child has an insulin requirement related to islet autoantibody development. All were weaned from pre-operative analgesic requirements. Gastric motility and glycaemic control post procedure were not significantly affected by lack of gastrostomy/gastro-jejunal feeding.

Conclusion: TP-IAT improves quality of life in Australian children with a significant burden of disease from chronic pancreatitis who have failed medical management. A national TP-IAT program should be established, with the procedure performed at sites with a concentration of expertise and experience.

Introduction

Total pancreatectomy and islet autotransplantation (TP-IAT) has been established as the surgery of choice for acute recurrent pancreatitis or chronic pancreatitis refractory to other therapies (1). The first TP-IAT undertaken in a child was described in 1996 (2), and further series demonstrated that the procedure improves quality of life in paediatric patients with severe chronic pancreatitis (3-6). Funding for islet transplantation in Australia is currently provided solely for the indication of hypoglycaemia unawareness complicating type 1 diabetes (7). Such allotransplantation carries risks associated with immunosuppression and rejection that are not seen with use of autologous islet tissue in TP-IAT. A collaborative initiative in South Australia has permitted the funding of a paediatric TP-IAT program at the Women's & Children's Hospital. We report the first five children who underwent TP-IAT in this program, all of whom had hereditary pancreatitis caused by a mutation in the PRSS1

(serine protease 1) cationic trypsinogen gene. These account for five of seven TP-IAT performed in Australia at the time of writing.

Patient cohort

The five children who underwent TP-IAT fulfilled published University of Minnesota criteria and recommendations from PancreasFest in 2014 (3,8). Patient one was a seven year nine month old boy of Aboriginal descent, with a history of frequent hospitalisations and background opioid requirement (9). He was known to have a PRSS1 mutation, as did his father and younger brother. Following the success of his procedure, three patients with a similarly severe burden of disease were independently referred for TP-IAT. Of interest, one of these patients was a distant relative of the index case. A fifth interstate patient with a PRSS1 mutation travelled to South Australia and underwent assessment and TP-IAT.

Patient age ranged from seven years and nine months, to seventeen years and ten months (table 1). Each had frequent hospitalisations prior to TP-IAT, ranging from 11-22 admissions for symptomatic relief. These, along with chronic symptoms impacted school attendance and quality of life. Three of the five had chronic narcotic requirements, including one who required background slow-release transdermal opioid relief. Another had input from a specialist pain service. All were prescribed pancreatic enzyme replacement to reduce pancreatic stimulation and treat suspected evolving exocrine insufficiency. No patients were known to be diabetic. Four out of five had a stimulated C-peptide level measured, confirming adequate beta cell function. One patient had a history of cyst jejunostomy for pseudocyst formation nine years prior, and two had had previous attempts at endoscopic duct decompression without sustained symptomatic improvement. Radiologic changes were abnormal in all pre-operatively, with features of atrophy, pancreatic calcification and/or duct dilatation.

Procedure

TP-IAT went ahead for each patient as a result of collaboration between multidisciplinary specialist teams. Consultation with overseas expertise was sought from Associate Professor Melena Bellin, University of Minnesota. The cost of the procedures was covered by our institution based on compassionate grounds, and by the relevant state health department for the interstate patient. Informed consent was obtained from families following extensive counselling with the transplant surgical, endocrinology and gastroenterology teams. Immunisation against encapsulated organisms via haemophilus, pneumococcus and meningococcal was provided pre-operatively for patients 1-4, and post-operatively in patient 5. All children had an early morning operative start. Each underwent total pancreatectomy, cholecystectomy and biliary and enteric reconstruction. Splenectomy was performed in three patients as a result of difficult dissection. Splenic tissue was reimplanted into the omentum after splenectomy in the index case. Feeding gastrostomy and/or gastrojejunostomy tubes were not sited, despite this being routine in North American centres (4,5).

Removed pancreatic tissue was placed in a modified University of Wisconsin solution omitting glutathione and glutamine. Tissue was maintained at four degrees Celsius during transport via commercial flight to Melbourne for islet isolation at St Vincent's institute of Medical Research. The average time elapsed from pancreatectomy to laboratory enzyme infusion was 304 minutes (range 255-387 minutes). Islet cell preparation included manual separation, enzymatic dissociation and utilisation of Ricordi digestion chamber. Microscopic examination of number of islets liberated was then performed, adopting the standardised islet mass unit; islet cell equivalents (IEQ), equating to an islet size of 150um diameter. IEQ ranged from 28,254 to 653,222 in our cohort (table 1). Tissue was then washed and suspended in a islet transplant specific Connaught Medical Research Laboratories (CMRL) medium, supplemented with 5% human serum albumin. Infusion bags were prepared, labelled and placed in sterile containers for immediate infusion on arrival back to transplant site. Sterility samples were taken from prepared media prior to transport.

Patients remained sedated in the Paediatric Intensive Care Unit (PICU) post pancreatectomy with intravenous glucose and insulin support until time of portal vein islet infusion. Time from start of pancreatectomy to infusion in the evening was 13 hours on average. Infusion was facilitated by radiologically guided percutaneous transhepatic puncture, with portal venous pressure monitoring. After TP-IAT, patients remained in PICU with strict blood glucose targets of 5.5- 6.6mmol/L achieved to maximise survival of transplanted islet cells. C-peptide levels were followed post-operatively. These varied between patients reflecting islet cell yield. Our first patient had the lowest IEQ infused,

and did not have a detectable C-peptide until day 15 post-operatively. Higher C-peptide levels were detected within days in the other patients (table 1).

Post-operative complications included ileus and pain. Two returned to theatre for laparotomies in the context of a bleeding splenic vein and a bowel obstruction from internal hernia. A suspected portal vein thrombosis was managed with anticoagulation, however this was never confirmed radiologically. This patient had late onset of gastrointestinal bleeding, found to be a result of a pyloroanastomotic ulcer that responded to protein pump inhibitor therapy. Two of our patients had post-operative dysmotility. One was managed medically with prokinetic therapy, and another ultimately required a period of jejunal feeding. In the latter patient, endoscopy revealed antral oedema felt to be in keeping with bile reflux gastropathy, which responded to a weaning course of steroids. This patient also had a late rise in transaminases in keeping with cholangitis, and was treated with antibiotic therapy. Two patients had bacterial growth on cultures from samples taken during the islet preparation process. Given one of these patients had a background of previous Puestow procedure, direct contamination may have resulted from distorted anatomy. Both affected recipients received susceptible antibiotics on return of gram stain and later culture, and had no sequelae from infected/contaminated graft tissue.

Duration of admission to PICU ranged from 6 to 12 days. Average transition from insulin infusion to continuous subcutaneous insulin infusion (640G Medtronic pump) and continuous glucose monitoring (Dexcom G5) occurred on day 12. Exocrine pancreatic insufficiency was managed with pancreatic enzyme supplementation with introduction of diet. Discharge home occurred after an average of 26 days in hospital. Patients were prescribed long term fat soluble vitamins and pancreatic enzyme supplementation. Prophylaxis for asplenia was prescribed for all patients who underwent splenectomy during TP-IAT, including the patient in whom splenic tissue had been re-implanted. Close endocrinology, gastroenterology and surgical follow up ensued.

Post TP-IAT all patients became independent of any opioid requirement. All resumed normal activities, including schooling and sport. Two patients have an ongoing insulin requirement long-term. This includes our first patient with the lowest islet cell yield, and another patient who developed islet autoantibody positive type 1 diabetes (T1D)after a short period of insulin independence. A third patient remains on exogenous insulin in the early months post procedure, with weaning requirements. One of the insulin independent patients (patient 2) has issues with hypoglycaemia refractory to feeding.

Discussion

The true incidence of paediatric chronic pancreatitis remains unknown, but it is probably an underreported phenomenon and cause of chronic abdominal pain in children (10). Chronic inflammatory cell infiltration and fibrosis of pancreatic tissue can result in exocrine and endocrine insufficiency with malabsorption and diabetes mellitus. In the adult population, alcohol and tobacco exposure are well-established risk factors for acute recurrent and chronic pancreatitis (11,12). Whilst obstructive factors, trauma, infection, metabolic disturbances, autoimmune disease, and toxins (including asparaginase, thiopurines and valproate) can be associated with childhood disease, genetic mutations are responsible for most (11,12).

Between 67- 75% children with chronic pancreatitis will have an identifiable pancreatitis-predisposing genetic mutation (11-13). By far, the most common is a mutation of the cationic trypsinogen gene, PRSS1. Such mutations cause failure of acinar cell feedback mechanisms, enhancing activation or preventing inactivation of acinar cationic trypsinogen production (14). This results in pancreatic autodigestion, inflammation and/or fibrosis (15,16). PRSS1 mutations have a high penetrance with clinical sequalae in more than 90% (12). A high likelihood of chronic pancreatitis is associated, along with a lifetime pancreatic cancer risk in excess of 40% (17,18). As such, it is one of the assessment parameters in the University of Minnesota criteria for TP-IAT referral (3). Children with PRSS1 mutations present at a young age (17), with first attack often less than age 7 (13). Of children with presenting with features of chronic pancreatitis before 6 years of age, PRSS1 mutations are seen in 43% (12). Gain of function mutations N29I and R122H account for 70% of PRSS1 mutations (16). These appear to exhibit more severe disease progression (12,17), and were accounted for in all five children in our cohort (table one). Other identifiable pancreatitis-predisposing genetic mutations involve the cystic fibrosis transmembrane regulator gene (CFTR), particularly in patients with a pancreatic sufficient phenotype. Loss of function mutations in genes that encode serine peptidase

inhibitor Kazal type 1 (SPINK1) and chymotrypsin C (CTRC) are associated. The latter appears to be associated with early onset of symptoms (11,12). Calcium-sensing receptor gene (CASR), Carboxypeptidase 1, Claudin 2, carboxyl ester lipase (CEL) and carboxyl ester lipase hybrid gene changes are more recently described associations (12).

Exclusion of other aetiologies for paediatric chronic pancreatitis should occur prior to any consideration of genetic work-up. The International study group of paediatric pancreatitis: in search for a cure (INSPPIRE) group consensus recommend genetic work-up to include PRSS1 gene mutation testing, sweat chloride testing or cystic fibrosis gene testing. Centre specific gene panels including SPINK1, CFTR and CTRC may identify risk factors for acute recurrent or chronic pancreatitis (19).

Chronic pancreatitis can cause significant disruption of childhood with frequent hospitalisation, school absence and inability to participate in daily activities. As such the cornerstone of management focuses on restoring quality of life. This is often achieved via narcotics and other analgesic approaches, including nerve block procedures. It is estimated one third of affected children will require narcotics to manage acute and chronic pain symptoms (19). Utilisation of pancreatic enzyme replacement is used to reduce pancreatic stimulation (11). Monitoring for fat-soluble deficiencies is also pertinent in suspected evolving exocrine insufficiency. Endoscopic interventions for troublesome chronic pancreatitis include decompression of the main pancreatic duct via endoluminal dilatation, stenting, stone retrieval or sphincterotomy (3,4). Traditionally, surgical interventions have included limited resection of a diseased gland or drainage procedures that include Puestow, Frey and Beger techniques (20,21). Total pancreatectomy and islet autotransplantation has emerged as a viable treatment option for acute recurrent or chronic pancreatitis refractory to medical and endoscopic therapies. Early referral in affected children should be considered, noting that previous surgical intervention (including the Puestow procedure), can negatively impact islet yield in subsequent TP-IAT (3-6,22). Distorted anatomy from previous surgery may also increase risk of infection, as observed in patient 2 (table one).

Young age appears to be a favourable prognostic factor for achieving insulin independence post TP-IAT (5). Larger paediatric series have shown more than 60% are insulin independent or require minimal exogenous insulin at 1 year (3), with rates as high as 80% in younger children aged 3-8 years (5). Overall, insulin independence has been seen in 40% paediatric patients at 10 years post TP-IAT (4). The individual yield of islets clearly plays a large role in each individual. An islet cell equivalent more than 2500 per kilogram of body weight is thought to correlate with better outcomes (4,5). In an adult case series, more than two thirds of patients achieved insulin independence at three years with an IEQ/kg >5000, up to one fifth with an IEQ/kg 2500-5000 and 12% in those with IEQ/kg <2500 (1). In our cohort, four of five had a promising islet yield ranging 7,299-11,665 IEQ/kg. Follow up revealed long term insulin independence in two of these patients (patient 2 and 4), with encouraging progress in a third (patient 5) who has declining requirements in the early months post procedure. Patient 4 became insulin independent 8 months post-operatively, but developed an insulin requirement associated with the development of glutamic acid decarboxylase islet autoantibodies some years later. Conversely our patient 1 had an IEQ/kg of 1,130 with a low islet yield reflected in delay in detectable c-peptide post-operatively, and long term insulin dependence. Refractory hypoglycaemia in patient 2 probably reflects successful engraftment but with a lack of the counter-regulatory hormones somatostatin and glucagon.

From large multi-centre paediatric series, TP-IAT is associated with improved scores on health-related quality of life questionnaires, including school attendance and reduced opioid requirements (4-6). This remained true regardless of ongoing insulin dependence (4). Narcotic independence in children post TP-IAT is seen in 50-80% at 1 year (3,5,23) with higher success in younger children less than 8 years (5) . Although formal quality of life assessments were not pursued in our cohort, each have no long-term opioid requirement, with reduced hospital admissions and restoration of daily function regardless of ongoing insulin requirements.

Delayed gastric emptying is reported to be a common phenomenon post TP-IAT (22). Many paediatric TP-IAT centres employ a feeding gastrostomy/gastro-jejunostomy tube to aid maintenance of glycaemic control and in anticipation of dysmotility (4,5). Without such an approach, we have been able to achieve tight glycaemic control in our cohort. Two of five patients displayed clinically significant symptoms of delayed gastric emptying. Whilst one required a period of nasojejunal

feeding, this was likely confounded by gastric antral oedema from bile reflux gastropathy causing a degree of outlet obstruction. As such, we do not believe gastrostomy or gastro-jejunostomy tube is a necessary adjunct in paediatric TP-IAT.

Five of the seven paediatric TP-IAT procedures performed in Australian children to date have been undertaken in Adelaide. This has been a considerable collaborative effort involving surgeons from the Women's & Children's Hospital and Flinders Medical Centre, South Australia; the islet transplant and interventional radiology service at the Royal Adelaide Hospital, South Australia; the gastroenterology, endocrinology and paediatric intensive care units at the Women's and Children's Hospital and the Islet Transplantation laboratory at the St Vincent's Institute of Medical Research, Victoria. Interstate transport for islet cell isolation alongside good operative planning has not proven an impediment to the procedure or outcome. We plan to perform all steps locally in the future. We believe this procedure is of great benefit to Australian children with a significant burden of disease from chronic pancreatitis. This procedure should be undertaken at sites appropriately equipped with a concentration of expertise and experience as part of a national TP-IAT program.

Words: 2478

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
PRSS1 mutation	p.N29I (heterozygous)	p.N29I (heterozygous)	p.R122H (heterozygous)	p.R122H (heterozygous)	p.N29I (heterozygous)
Family history of PRSS1 mutation	Father and brother	Father and brother	Mother and two siblings and mother	Mother (required Whipple's and partial pancreatectomy), younger brother, maternal grandfather	Mother
Age of first documented episode	3 years 11 months	1 year 4 months	10 years 4 months	1 year 10 months	2 years 6 months
Number of pancreatitis hospital admissions prior to TP-IAT	14	22	13	Unknown	11
Age at time of TP-IAT	7 years 9 months	17 years 10 months	17 years 10 months	8 years 1 month	16 years 8 months
Date of procedure	14/7/15	2/8/17	12/4/17	25/7/19	9/1/20
Previous operations/ relevant procedures	3 x previous ERCP duct stenting procedures	Cyst jejunostomy (Puestow procedure) for pseudocyst age 8. Appendicectomy age 17 years 1 month.	Nil	Nil	ERCP and pancreatic sphincterotomy
Pre-operative oral glucose tolerance test +/- stimulated c-peptide	Fasting glucose 2.1 mmol/L, with glucose 2.8mmol/L at 120 minutes	Fasting glucose 4.0mmol/L (c-peptide 420pmol/L) with glucose 3.9mmol/L at 120 minutes	Fasting glucose 4.6mmol/L (c-peptide 381pmol/L) with glucose 6.9mmol/L at 120 minutes	Fasting glucose 5.0 (C-peptide 779pmol/L) with glucose 4.9mmol/L at 120 minutes	Fasting glucose 5.0 (c-peptide 779pmol/L), with glucose 4.9mmol/L at 120 minutes
Pre-op pancreatic enzyme therapy	Creon	Creon	Creon	Pancreozystat	Creon
Pre-op imaging changes	Dilated pancreatic duct, atrophy of pancreatic tissue, calcifications in body and tail	Significant dilatation of the main pancreatic duct	Mild prominence pancreatic duct	Reduction in pancreatic bulk. Dilated and tortuous pancreatic duct	Calcification with pancreatic stone
Pre-operative narcotic dependence	Amitriptyline regularly, oxycodone 4mg daily	Buprenorphine patches 5 microg/hour. Oxycodone 10mg per day. Pregabalin 25mg BD.	Oxycodone 5mg twice daily	Pregabalin, nil opioid between attacks, specialist pain clinic involvement	Panadeine forte
Pre-operative impaired QOL (school attendance, prolonged frequent admissions)	Impact on school attendance, frequent prolonged admissions	Impact on school attendance, significant chronic pain	Impact on school attendance, frequent prolonged admissions	Impact on school attendance (>6 weeks/year), frequent prolonged admissions	Impact on school attendance
Pre-op vaccination against encapsulated organisms	Yes	Yes	Yes	Yes	No, received post-operatively
Splenectomy undertaken during TP-IAT	Yes. Splenic remnants subsequently reimplanted into mesentery	No	No	Yes	Yes
Minutes from pancreatectomy to laboratory enzyme perfusion	280	387	300	300	255
Time from pancreatectomy to	11	16	12	13	13

islet cell infusion (hours)					
Body weight (kg)	25	56	67	26.4	68
Pancreas weight (g)	25	80	42.3	45.4	66.5
Islet cell equivalents (IEQ)	28,254	653,222	489,000	302,458	557,036
IEQ per gram pancreas	1,130	9,749	13,852	7,561	9,571
IEQ per recipient kg weight	1,130	11,665	7,299	11,457	8,192
PICU duration (days)	7	7	6	12	12
Post-operative complications	- Suspected portal vein thrombosis. - Bleeding pyloroanastomotic ulcer causing haemoglobin drop day 54.	- Repeat laparotomy for splenic vein bleeding day 2 - Growth Escherichia Coli islet cell culture: 9 days intravenous piperacillin/tazobactam	- Nil	- Small bowel obstruction secondary to internal hernia (small bowel omentum) with relook laparotomy/ reduction day 26 post-op. - Delayed gastric emptying: brief use of erythromycin with response	- Perihepatic haematoma day 1 post operatively requiring blood transfusion. - Growth of graniculatella species in islet cell culture: 7 days intravenous piperacillin/tazobactam - Post-operative ileus + gastroparesis; transpyloric tube sited day 14, reflux gastropathy with antral oedema, responded to weaning steroid course. - Deranged liver function tests: treated for cholangitis 7 days
Day post TP-IAT transitioned from insulin infusion to subcutaneous insulin pump	13	12	14	12	12
Earliest detectable c-peptide post-operatively	0.05 (day 15) 203 (8 weeks post-op) 490 (13 months post-op)	2099 (day 2)	1808 (day 5)	428 (day 2)	703 (day 2)
Long term insulin dependence	Yes	No (Note: hypoglycaemia refractory to feeds)	Yes: initially weaned (8 months post-operatively); developed anti-GAD antibody positiveT1D 20 months post TP-IAT	No	0.12 unit/kg/day 5 months post-operatively
Long term opioid requirement	Nil	Nil	Nil	Nil	Nil
Long term pancreatic /fat soluble vitamin supplementation	Creon + ABDECK	Creon + ABDECK	Creon Fat soluble vitamin levels normal post-op	Pancreozystat, ABDECK	Creon, ABDECK
Length of stay in hospital (days)	31	15	20	35	30

References

1. Sutherland DE, Radosevich DM, Bellin MD, et al. Total pancreatectomy and islet autotransplantation for chronic pancreatitis. J Am Coll Surg 2012;214:409–424; discussion 424–426.
2. Wahoff DC, Paplois BE, Najarian JS, Farney AC, Leonard AS, Kendall DM, et al. Islet autotransplantation after total pancreatectomy in a child. J Pediatr Surg 1996;31:132-5.
3. Bellin MD, Freeman ML, Schwarzenberg SJ, et al. Quality of life improves for pediatric patients after total pancreatectomy and islet autotransplant for chronic pancreatitis. Clin Gastroenterol Hepatol. 2011;9:793–799.
4. Chinnakotla S, Bellin MD, Schwarzenberg SJ, et al. Total pancreatectomy and islet autotransplantation in children for chronic pancreatitis: indication, surgical techniques, postoperative management, and long-term outcomes. Ann Surg. 2014;260:56–64.
5. Bellin MD, Forlenza GP, Majumder K, et al. Total pancreatectomy with islet autotransplantation resolves pain in young children with severe chronic pancreatitis. J Pediatr Gastroenterol Nutr 2017;64:440–445
6. Wilson GC, Sutton JM, Salehi M, et al. Surgical outcomes after total pancreatectomy and islet cell autotransplantation in pediatric patients. Surgery 2013; 154:777–783; discussion 783–784.
7. Webster AC, Hedley JA, Anderson PF, Hawthorne WJ, Radford T, Drogemuller C, Rogers N, Goodman D, Lee MH, Loudovaris T, Kelly PJ. Australia and New Zealand Islet and Pancreas Transplant Registry Annual Report 2018-Islet Donations, Islet Isolations, and Islet Transplants. Transplant Direct. 2019 Jan 7;5(2):e421.
8. Bellin MD, Freeman ML, Gelrud A, et al. Total pancreatectomy and islet autotransplantation in chronic pancreatitis: recommendations from PancreasFest. Pancreatology. 2014;14(1):27-35.
9. Geyer M, Coates P, Khurana S, Chen J, Kay T, Balamurugan A, Couper J, Radford T, Drogemuller C, Loudovaris T, Pathi R. First Report of Successful Total Pancreatectomy and Islet Autotransplant in Australia. Pancreas. 2017;46(3).
10. Nydegger A, Couper RT, Oliver MR. Childhood pancreatitis.J Gastroenterol Hepatol. 2006;21:499-509
11. Uc A, Husain SZ. Pancreatitis in Children. Gastroenterology. 2019;156(7): 1969-1978
12. Giefer MJ, Lowe ME, Werlin SL, et al. Early-Onset Acute Recurrent and Chronic Pancreatitis Is Associated with PRSS1 or CTRC Gene Mutations. J Pediatr. 2017;186:95-100.
13. Schwarzenberg SJ, Bellin M, Husain SZ, et al. Pediatric chronic pancreatitis is associated with genetic risk factors and substantial disease burden. J Pediatr 2015; 166:890–896 e1.
14. Gorry MC, Gabbaizedeh D, Furey W et al. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. Gastroenterology 1997; 113: 1063–8.
15. Whitcomb DC, Gorry MC, Preston RA et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat. Genet. 1996; 14: 141–5.
16. Sutton JM, Schmulewitz N, Sussman JJ, Smith M, Kurland JE, Brunner JE, et al. Total pancreatectomy and islet cell autotransplantation as a means of treating patients with genetically linked pancreatitis. Surgery 2010;48:676-85.
17. Rebours V, Boutron-Ruault MC, Schnee M, Ferec C, Le Marechal C, Hentic O, et al. The natural history of hereditary pancreatitis: a national series. Gut 2009;58:97-103.
18. Howes N, Lerch MM, Greenhalf W, et al. Clinical and genetic characteristics of hereditary pancreatitis in Europe. Clin Gastroenterol Hepatol. 2004;2:252–261.
19. Gariepy CE, Heyman MB, Lowe ME, et al. Causal evaluation of acute recurrent and chronic pancreatitis in children: consensus from the INSPPIRE group. J Pediatr Gastroenterol Nutr 2017;64:95–103.
20. Clifton MS, Pelayo JC, Cortes RA, et al. Surgical treatment of childhood recurrent pancreatitis. J Pediatr Surg. 2007;42:1203–1207.
21. Iqbal CW, Moir CR, Ishitani MB. Management of chronic pancreatitis in the pediatric patient: endoscopic retrograde cholangiopancreatography vs operative therapy. J Pediatr Surg. 2009;44:139–143.
22. Kesseli SJ, Smith KA, Gardner TB. Total pancreatectomy with islet autologous transplantation: the cure for chronic pancreatitis?. Clin Transl Gastroenterol. 2015;6(1):e73.
23. Chinnakotla S, Radosevich DM, Dunn TB, et al. Long-term outcomes of total pancreatectomy and islet auto transplantation for hereditary/genetic pancreatitis. J Am Coll Surg 2014;218:530–43.

2. Tables

Table 1 – University of Minnesota Criteria for total pancreatectomy and islet auto transplantation (46)

Table 1: University of Minnesota Criteria[163]
To be considered for TPIAT, patients must meet criteria in sections I and II and have no contraindications:

I. Definitions (must have one of the following: a, b, or c)

a. CP (must have one of i, ii, or iii) Patients with chronic abdominal pain, lasting > 6 months, features consistent with that of pancreatitis, and evidence of CP as evidenced by at least one of the following:
   - i. Morphologic/functional evidence of CP [CT of abdomen with evidence of CP (calcifications), or ERCP evidence of pancreatitis]
   - *or*
   - ii. EUS of ≥ 6/9 criteria positive of CP
   - *or*
   - iii. At least 2 of the following 3 findings:
     1. Secretin MRCP or ERCP, with findings suggestive of CP (abnormal duct/side branch) or MRI T2 evidence of fibrosis
     2. EUS with ≥ 4/9 criteria positive for pancreatitis
     3. Abnormal exocrine pancreatic function tests (peak bicarbonate < 80)

   or

b. Relapsing AP (must have both 1 and 2)
   - i. Three or more episodes of documented AP with ongoing episodes over > 6 months.
   - ii. No evidence of current gallstone disease or other correctable etiology such as autoimmune pancreatitis

   or

c. Documented hereditary pancreatitis with compatible clinical history.

II. Indications for TPIAT (must have each of the following: 1-5)

a. Documented CP or relapsing AP with chronic or severe abdominal pain, directly resulting in at least one of the following:
   - i. Chronic narcotic dependence (patient requires narcotics on a daily or nearly daily basis for > 3 months)
   - ii. Impaired quality of life, defined by at least one of the following:
     1. Loss of job
     2. Inability or significantly reduced ability to work or attend school
     3. Frequent absences from school
     4. Frequent hospitalizations
     5. Loss of ability to participate in usual age-appropriate activities
   - iii. Complete evaluation, with no reversible cause of CP or relapsing AP present or untreated
   - iv. Unresponsive to maximal medical therapy and endoscopic therapy, with ongoing abdominal pain requiring routine narcotics for CP or relapsing AP
   - v. Adequate islet cell function (non-diabetic, or non-insulin-requiring diabetes with C-peptide positive)

Table 2 – CRediT – Contributor Roles Taxonomy (48)

TABLE 1 Contributor Roles Taxonomy (CRediT).

Term	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components
Validation	Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/experiments and other research outputs
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyse or synthesize study data
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
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
Writing – original draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)
Writing – review and 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
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/data presentation
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team
Project administration	Management and coordination responsibility for the research activity planning and execution
Funding acquisition	Acquisition of the financial support for the project leading to this publication

Table 3 – Causes of Paediatric non-syndromic bile duct paucity (57)

Non-syndromic bile duct paucity	
Metabolic and genetic disorders	Alpha-1 antitrypsin deficiency
	Progressive familial intrahepatic cholestatic disorder
	HNF3β deficiency
	GLIS3 mutations
	Cystic fibrosis
	Peroxisomal disorders
	Niemann pick type C
	ARC syndrome
	Kabuki syndrome
	Trisomy 17, 18 and 21
Infections	Congenital syphilis
	Congenital rubella
	Congenital cytomegalovirus
Inflammatory and immune disorders	Graft versus host disease
	Chronic hepatic graft rejection
	Sclerosing cholangitis
	Advanced biliary atresia
	Sarcoidosis
Other	Drug induced, vanishing bile duct syndrome
	Panhypopituitarism
	Idiopathic

Table 4 - Roussel Uclaf Causality Assessment Method (72)

Time to onset	Cholestatic or Mixed Type	Assessment
From the beginning of the drug		
• Suggestive	5~90 days	+2
• Compatible	<5 or >90 days	+1
Course	**Change in alk phos (or total bilirubin) between peak value and ULN**	**Score**
After stopping the drug		
Highly suggestive	Not applicable	+3
Suggestive	Decrease ≥50% within 180 days	+2
Compatible	Decrease <50% within 180 days	+1
Inconclusive	Persistence or increase or no information	0
Against the role of the drug	Not applicable	-2
Risk Factors	**Ethanol or pregnancy**	**Score**
Alcohol or pregnancy	Presence	+1
	Absence	0
Age		
	Age of the patient ≥55 years	+1
	Age of the patient <55 years	0
Concomitant drugs		**Score**
None or nor information or concomitant drug incompatible time to onset		0
Concomitant drug with suggestive or compatible time to onset		-1
Concomitant drug known to be hepatoxic with a suggestive time to onset		-2
Concomitant drug with clear evidence for its role		-3
Exclusion of other causes of liver injury		**Score**
Group I (6 causes):		
• Acute viral hepatitis due to HAV or HBV or HCV	• All causes in Group I and II ruled out	+2
• Biliary obstruction (by imaging)	• The 6 causes of Group I ruled out	+1
• Alcoholism	• Five or 4 causes of Group I ruled out	0
• Recent history of hypotension, shock or ischemia	• Less than 4 causes of Group I ruled out	-2
Group II (categories of causes)	• Non drug cause highly probable	-3
• Complications of underlying diseases such as autoimmune hepatitis, sepsis, chronic hepatitis B or C, primary biliary cirrhosis or sclerosing cholangitis or		
• Clinical features or serologic and virologic tests indicating acute CMV, EBV or HSV		
Previous information on hepatotoxicity of the drug		**Score**
Reaction labeled in the product characteristics		+2
Reaction published but unlabeled		+1
Reaction unknown		0
Response to readministration		**Score**
Positive	Doubling of Alk P (or bilirubin) with drug alone	+3
Compatible	Doubling of the Alk P (or bilirubin) with the suspect drug combined with another drug which had been given at the time of onset of the initial injury	+1
Negative	Increase of Alk P (or bilirubin) but less than LUN with drug alone	-2
Not done or not interpretable	Other situations	0
Initial ALT: 96 units/L Initial Alkaline Phosphatase (Alk P) units/L: 359 R ratio: [ALT/ULN] / [Alk P/ULN] = [96/35] / [359/150] = 1.1 The R ratio determines whether the injury is hepatocellular (R>5), cholestatic (R<2), or mixed (R 2-5)		

Table 5 – Known DILI associated HLA alleles with associated drugs (130–143)

HLA Allele	Associated drugs
HLA -B*57:01	Abacavir
HLA-B*39:01 HLA-A*34:02 HLA-B*58:01	Allopurinol
DRB1*15:02 DRB5*0101 DQB1*0602 HLA-A*02:01 DRB1*15:01	Amoxicillin / Clavulanate
HLA-B*57:02 HLA-B*57:03	Anti-HIV and Anti-TB drug combinations
HLA-A*31:01 HLA-B*15:02	Carbamazepine
HLA-B*13:01	Dapsone
DRB1*04:03 HLA-B*35:03	Diclofenac
HLA-B*57:01 HLA-B*57:03 HLA-C*04:01 HLA-DRB1*01	Flucloxacillin
HLA-DRB1*16:01	Flupirtine
HLA-B*35:01	Green tea
	Polygonum multiforum
HLA-B*39:01	Infliximab
HLA-B*52:01	Isoniazid
HLA-DRB1*07:01	Lapatinib Ximelagatran
HLA-DRB1*15:01	Lumiracoxib
HLA-B*35:02	Minocycline
HLA-C*03:02	Methimazole
HLA-DRB1*01:01 HLA-DRB1*01:02 HLA-B*58:01 HLA-C*04:01 HLA-B*35:05	Nevirapine
HLA-DRB1*11:04	Nitrofurantoin
HLA-B*53:01	Phenytoin
HLA-B*57:01	Pazopanib
HLA-A*33:01	Terbinafine Ticlopidine Fenofibrate
HLA-A33:03	Ticlopidine
HLA-B*14:01-C*08:02	Trimethoprim - Sulfamethoxazole

Table 6 – King's College Criteria for Acetaminophen Toxicity (76)

King's College Criteria
The presence of one of the following should prompt a referral/transfer to a liver transplantation center:
Acidosis (admission arterial pH < 7.30) **OR**
Hepatic encephalopathy (grade III or IV), AND coagulopathy (PT > 100 s), AND acute kidney injury (creatinine > 3.4 mg/dL), **OR**
Hyperlactatemia (4-hour lactate > 3.5 mmol/L, or 12-hour lactate > 3.0 mmol/L), **OR**
Hyperphosphatemia (48-96 hour phosphate > 3.7 mg/dL) in patients with acetaminophen-induced fulminant hepatic failure.

Table 7 – Liver disease specific organoids (109)

Organoid type	Cell source	Disease	Modeling approach		References
			Patient-derived	Genome editing	
Hepatic	Liver Biopsy	A1-Antitrypsin Deficiency, Alagille Syndrome	✓		Huch et al., 2015
Tumor	Surgical Resection	Liver Cancer	✓		Broutier et al., 2017
Tumor	Needle Biopsy	Liver Cancer	✓		Nuciforo et al., 2018
Tumor	Surgical Resection	Liver Cancer	✓		Li et al., 2019
Hepatic	iPSCs	Alagille Syndrome	✓	✓	Guan et al., 2017
Hepatic	iPSCs	Citrullinemia type I	✓		Akbari et al., 2019
Hepatic	iPSCs	Steatohepatitis Wolman Disease	✓		Ouchi et al., 2019
Cholangiocyte	iPSCs	Cystic Fibrosis	✓		Ogawa et al., 2015
Cholangiocyte	iPSCs	Cystic Fibrosis	✓		Sampaziotis et al., 2015

Table 8 – Liver directed gene therapy clinical trials (113)

Table 1 | Liver-directed gene therapy clinical trials

Indication	Therapeutic agent	Trial phase (status)	Available outcomes	Refs.
Haemophilia B	ssAAV2-F9WT	Phase I–II (terminated)	Successful liver transduction; elevation of transaminases, leading to loss of expression in patients who received high doses; rAAV neutralization by antibody titre above 1:5	92,224
	scAAV8-F9WT	Phase I (not recruiting, active)	Long-term therapeutic factor IX expression associated with clinical improvement; alanine aminotransferase rise, controlled after prednisolone treatment; no late toxic effects reported	93,225,226
	ssAAV5-coF9WT	Phase I–II (completed)	With low dose, annualized factor IX use reduced by 81%, mean ASBR reduced by 53%; with high dose, annualized factor IX use reduced by 73%, mean ASBR reduced by 70%	227,228
	ssAAV.SPARK100-F9Padua (SPK-9001)	Phase I–II (completed)	Sustained therapeutic expression of factor IX coagulant activity after gene transfer in 10 participants; termination of baseline prophylaxis and the near elimination of bleeding and factor use; no serious adverse events during or after vector infusion	94
		Phase III (active)	Ongoing	50
	scAAV8-F9Padua (BAX 335)	Phase I–II (active)	Sustained therapeutic factor IX activity of ~20%, without bleeding or replacement therapy, for 4 years in 1 patient; corticosteroid treatment did not stabilize factor IX activity loss; 4 serious adverse events in 3 participants, all considered unrelated to BAX 335; no deaths	95,229
	ssAAV5-coF9Padua (AMT-061)	Phase IIb (active)	Mean factor IX activity of 31% at week 6, increasing to 47% at 26 weeks; sustained activity of >40% in 2 participants; associated with complete bleed cessation with no need for factor IX replacement therapy up to 26 weeks	47,230
	ssAAVS3-coF9Padua (FLT180a)	Phase I–II (terminated)	Factor IX activity levels ≥50% in 7 of 8 patients treated with the three highest doses; normal levels of factor IX activity achieved with relatively low vector doses; loss of transgene expression early owing to transaminitis in 1 patient	231,232
	ssAAVrh10-coF9WT (DTX-101)	Phase I–II (terminated)	Improved levels of factor IX in all patients during post-treatment follow-up; increased ALT levels in 5 of 6 patients; trial was discontinued	233,234
Haemophilia A	ssAAV5-coBDDF8 (BMN-270)	Phase I–II (active)	Transgene expression and haemostatic response for up to 5 years; most common adverse events associated with the treatment were transient, asymptomatic mild-to-moderate ALT elevations	55,235
	ssAAVLK03-coBDDF8 (SPK-8011)	Phase I–II (recruiting, active)	Sustained factor VIII expression in 16 of 18 participants who received SPK-8011 permitted discontinuation of prophylaxis and a reduction in bleeding episodes; some participants received glucocorticoids within 52 weeks after vector administration to prevent or treat a presumed AAV capsid immune response; 17 vector-related adverse events, including 1 serious, and 16 glucocorticoid-related adverse events	165,236
	ssAAV6-coBDDF8 (PF-07055480)	Phase I–II	Increases in factor VIII levels in the mild-to-normal range, with sustained bleeding control; generally well tolerated; most commonly reported treatment-related adverse events included elevated liver enzymes and infusion-related reactions; treatment-related serious adverse events reported in 1 patient	237
		Phase III (recruiting, active)	Ongoing	53
	ssAAVhu37-coBDDF8 (DTX-201)	Phase I–II (active)	Sustained factor VIII levels (≥5%) for up to 16 months in 5 of 6 patients; all patients in two cohorts off prophylaxis since ~6 weeks after gene transfer; no serious adverse events were reported before 2020	238,239
	ssAAV8-coBDDF8 (TAK-754)	Phase I–II (active)	Factor VIII activity peaked 4–9 weeks after infusion but declined during tapering of corticosteroids; factor VIII prophylaxis resumed in 2 of 4 patients; minor transaminase elevation	240
Ornithine transcarbamylase deficiency	scAAV8-OTC (DTX301)	Phase I–II (completed)	Ammonia control maintained or improved in all 9 treated patients; 3 patients who received the highest dose were considered complete responders and have discontinued alternative medications and protein-restricted diets without loss of ammonia control; elevations of transaminases were controlled with steroid-reactive treatment	63,241

Table 1 (Continued) | Liver-directed gene therapy clinical trials

Indication	Therapeutic agent	Trial phase (status)	Available outcomes	Refs.
Phenylketonuria	ssAAVHSC15-PAH (HMI-102)	Phase I–II (recruiting)	Two dose levels were generally well tolerated and led to clinically meaningful reductions in phenylalanine levels, increases in tyrosine and reductions in the phenylalanine to tyrosine ratio	65,242
	ssAAV5-PAH (BMN 307)	Phase I–II (on hold)	Trial placed on clinical hold owing to potential drug genotoxicity in a mouse preclinical study; the clinical relevance remains under investigation	243,244
	ssAAVHSC15-PAH homology arms (HMI-103)	Phase I (recruiting)	No data available	245
Acute intermittent porphyria	ssAAV5-coPBGD	Phase I (completed)	Partial symptomatic relief and a good safety profile but no reduction in porphyrin precursor levels	157,246
Methylmalonic acidaemia	ssAAVLK03-MMA integrative (hLB-001)	Phase I–II (recruiting, active)	No drug-related serious adverse events were reported in the first 2 patients but thrombotic microangiopathy developed in the subsequent, younger 2 patients	123,247
Familial hypercholesterolaemia	ssAAV8-hLDLR (RGX-501)	Phase I–II (completed)	No data available	248
Glycogen storage disease type 1a	ssAAV8-G6PC (DTX-401)	Phase I–II (completed)	Significant reductions in the need for cornstarch and improvements in glucose control and other metabolic parameters compared to baseline in all 9 patients	68,249
Wilson disease	ssAAV3B-ATP7B (VTX-801)	Phase I–II (recruiting)	No data available	250
	ssAAV9-ATP7B (UX701)	Phase I–II (recruiting)	No data available	251
Crigler–Najjar syndrome	ssAAV8-UGT1A1 (GNT0003)	Phase I–II (recruiting)	Temporary therapeutic effect in patients who received the lowest dose; significant reduction in bilirubin levels in patients treated with a higher dose; 2 patients stopped phototherapy, a third is under evaluation; good safety and tolerability	69,70
Hereditary transthyretin amyloidosis	LNP-CRISPR–Cas9 targeting *TTR* gene	Phase I (recruiting, active)	Decreases in serum TTR protein concentrations in the 6 patients treated; 96% reduction in TTR in those who received higher doses; only mild adverse events	134,252

AAV, adeno-associated virus; ALT, alanine transaminase; ASBR, annualized spontaneous bleeding rate; LNP, lipid nanoparticle; rAAV, recombinant AAV; sc, self-complementary; ss, single-stranded; TTR, transthyretin.

2. Letters of Support for Publications Included in Thesis

To Higher Degrees by Research office, Flinders University,

As the first author of the paper "Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause" I give consent for it to be included in the main body of his PhD thesis.

This paper was published in the New England Journal of Medicine, the most prestigious and impactful journal for clinical medicine, the area in which Michael is writing his thesis. Publications of this magnitude in clinical medicine typically have several authors making a large contribution to the publication. Michael made a significant and large contribution to this publication as second author in terms of collating the patient data, co-writing the initial manuscript, and drawing up the tables and figures. I therefore wholeheartedly support him including it in the body of his PhD thesis.

Should you have any queries or concerns please do not hesitate to get in touch.

Yours sincerely,

Chayarani Kelgeri,

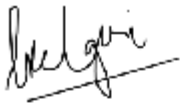
Paediatric Hepatologist,

Liver Unit, Birmingham Children's Hospital, UK.

To Higher Degrees by Research office, Flinders University,

As the first author of the paper "Aetiology, Characteristics and Outcomes of Neonatal Liver Failure: lessons learned over the last 3 decades" I confirm Michael Couper contributed to this publication and I give consent for it to be included in the appendix of his PhD thesis.

Yours sincerely,



Chayarani Kelgeri,

Paediatric Hepatologist,

Birmingham Children's Hospital, Liver Unit

To Higher Degrees by Research office, Flinders University,

As the first author of the paper "South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1-associated hereditary pancreatitis" I confirm Michael Couper contributed to this publication and I give consent for it to be included in the appendix of his PhD thesis.

Yours sincerely,

Jessica Eldredge,

Paediatric Gastroenterologist,

MBBS, FRACP

AUTHOR BIBLIOGRAPHY

1 Philpott, Hamish & Lemberg, Daniel & Day, Andrew & Rosenbaum, Jeremy & Singh, Harveen & Rumore, Sarah & Ellison, Samuel & Couper, Michael & Porter, Jody & Roberts, Amin & Thacker, Kunal & Moore, David & Furata, Glenn & Sharma, Ajay. (2024). Characteristics and management of eosinophilic esophagitis in Australasian children: a decade of experience. Internal Medicine Journal. 10.1111/imj.16558.

2 Kelgeri C, Kanthimathinathan HK, Couper M, Alnagar A, Biradar V, Sharif K, Hartley J, Mirza D, Gupte GL. Etiology, Characteristics, and Outcomes of Neonatal Liver Failure: Lessons Learned Over the Last 3 Decades. J Pediatr. 2024 Aug 14;275:114245. doi: 10.1016/j.jpeds.2024.114245. Epub ahead of print. PMID: 39151605.

3 Couper MR, Brown RM, Nath S, Parida A, Kelgeri C. Periportal necrosis and successful liver transplantation following Lamotrigine drug-induced liver injury in a child. BMJ Case Rep. 2023 Nov 24;16(11):e255787. doi: 10.1136/bcr-2023-255787. PMID: 38000812; PMCID: PMC10679976.

4 Couper MR, Valamparampil J, Thyagarajan M, Hartley J, Gupte G. Intestinal ultrasound may be a useful tool in monitoring acute rejection following intestinal transplantation. Pediatr Transplant. 2023 Sep;27(6):e14574. doi: 10.1111/petr.14574. Epub 2023 Jul 17. PMID: 37458363

5 Couper MR, Brown RM, Gupte G, Perera MTPR, Kelgeri C. Liver Disease in GLIS3 Mutations: Transplant Considerations and Bile Duct Paucity on Explant Histology. J Pediatr Gastroenterol Nutr. 2023 Jul 1;77(1):110-114. Epub 2023 Mar 14. PMID: 36917836.

6 Kelgeri C, Couper M, Gupte GL, Brant A, Patel M, Johansen L, Valamparampil J, Ong E, Hartog H, Perera MTPR, Mirza D, Van Mourik I, Sharif K, Hartley J. Clinical Spectrum of Children with Acute Hepatitis of Unknown Cause. N Engl J Med. 2022 Jul 13.

7 Couper MR, Eldredge JA, Kirby M, Kirby C, Moore D, Hammond P, Manton N, Glynn A, Couper RT. Paediatric Gastrointestinal, Hepatic and Pancreatic inflammatory Myofibroblastic Tumours, A single center experience. J Pediatr Gastroenterol Nutr. 2022 Feb 1;74(2):253-257.

8 Couper MR, Shun A, Siew S, O'Loughlin E, Thomas G, Andersen B, Jermyn V, Sawyer J, Stormon MO. Pediatric third liver transplantation-A single-center experience. Pediatr Transplant. 2021 ec;25(8):e14092.

9 Eldredge J, Couper MR, Moore DJ, Khurana S, Chen JW, Couper JJ, Drogemuller CJ, Radford T, Kay TW, Loudovaris T, Wilks M, Coates PT, Couper RT. South Australian experience with paediatric total pancreatectomy and islet autotransplantation for PRSS1-associated hereditary pancreatitis. Med J Aust. 2021 Oct 4;215(7):294-296.e1.

10 Couper MR, Chennapragada M, Magoffin A. Hepatobiliary and Pancreatic: A rare peribiliary lesion. J Gastroenterol Hepatol. 2021 Sep;36(9):2336. doi: 10.1111/jgh.15556. Epub 2021 Jun 29.

11 Eldredge JA, Couper MR, Barnett CP, Rawlings L, Couper RTL. New Pathogenic Mutations Associated with Diacylglycerol O-Acyltransferase 1 Deficiency. J Pediatr. 2021 Jun;233:268-272. doi: 10.1016/j.jpeds.2021.02.028. Epub 2021 Feb 17.

REFERENCES

1. Spada M, Riva S, Maggiore G, Cintorino D, Gridelli B. Pediatric liver transplantation. World J Gastroenterol. 2009 Feb 14;15(6):648–74.

2. World Health Organisation. WHO. 2022. World Health Organization. Acute hepatitis of unknown aetiology in children — multicountry (https://www.who.int/emergencies/ disease-outbreak-news/item/DON-389).

3. Bowring MG, Massie AB, Chu NM, Bae S, Schwarz KB, Cameron AM, et al. Projected 20- and 30-Year Outcomes for Pediatric Liver Transplant Recipients in the United States. J Pediatr Gastroenterol Nutr. 2020 Mar;70(3):356–63.

4. Bailey B, Amre DK, Gaudreault P. Fulminant hepatic failure secondary to acetaminophen poisoning: a systematic review and meta-analysis of prognostic criteria determining the need for liver transplantation. Crit Care Med. 2003 Jan;31(1):299–305.

5. Katarey D, Verma S. Drug-induced liver injury. Clinical Medicine. 2016 Dec;16(6):s104–9.

6. Jeffrey AW, Jeffrey GP, Stormon M, Thomas G, O'Loughlin E, Shun A, et al. Outcomes for children after second liver transplantations are similar to those after first transplantations: a binational registry analysis. Med J Aust. 2020 Nov;213(10):464–70.

7. Kaufman SS, Atkinson JB, Bianchi A, Goulet OJ, Grant D, Langnas AN, et al. Indications for pediatric intestinal transplantation: a position paper of the American Society of Transplantation. Pediatr Transplant. 2001 Apr;5(2):80–7.

8. Rumbo M, Oltean M. Intestinal Transplant Immunology and Intestinal Graft Rejection: From Basic Mechanisms to Potential Biomarkers. Int J Mol Sci. 2023 Feb 25;24(5).

9. Gurkan A. Advances in small bowel transplantation. Turk J Surg. 2017 Sep 8;33(3):135–41.

10. Kilgore A, Mack CL. Update on investigations pertaining to the pathogenesis of biliary atresia. Pediatr Surg Int. 2017 Dec;33(12):1233–41.

11. The NS, Honein MA, Caton AR, Moore CA, Siega-Riz AM, Druschel CM, et al. Risk factors for isolated biliary atresia, National Birth Defects Prevention Study, 1997-2002. Am J Med Genet A. 2007 Oct 1;143A(19):2274–84.

12. Girard M, Jannot AS, Besnard M, Jacquemin E, Henrion-Caude A. Biliary atresia: does ethnicity matter? J Hepatol. 2012 Sep;57(3):700–1; author reply 702.

13. Fawaz R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL, et al. Guideline for the Evaluation of Cholestatic Jaundice in Infants: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2017 Jan;64(1):154–68.

14. Cortes-Cerisuelo M, Boumpoureka C, Cassar N, Joshi D, Samyn M, Heneghan M, et al. Liver Transplantation for Biliary Atresia in Adulthood: Single-Centre Surgical Experience. J Clin Med. 2021 Oct 26;10(21).

15. Guandalini S, Dhawan A, editors. Textbook of Pediatric Gastroenterology, Hepatology and Nutrition. Cham: Springer International Publishing; 2022.

16. Zeng DY, Li JM, Lin S, Dong X, You J, Xing QQ, et al. Global burden of acute viral hepatitis and its association with socioeconomic development status, 1990-2019. J Hepatol. 2021 Sep;75(3):547–56.

17. Kiyasu PK, Caldwell SH. Diagnosis and treatment of the major hepatotropic viruses. Am J Med Sci. 1993 Oct;306(4):248–61.

18. World Health Organisation. WHO. 2023. Hepatitis E - Fact sheet (https://www.who.int/news-room/fact-sheets/detail/hepatitis-e).

19. Kelly D. Viral hepatitis B and C in children. J R Soc Med. 2006 Jul;99(7):353–7.

20. Stinco M, Rubino C, Trapani S, Indolfi G. Treatment of hepatitis B virus infection in children and adolescents. World J Gastroenterol. 2021 Sep 28;27(36):6053–63.

21. World Health Organisation. WHO. 2024. Hepatitis C - Fact sheet (https://www.who.int/news-room/fact-sheets/detail/hepatitis-c#:~:text=Direct%2Dacting%20antiviral%20medicines%20(DAAs,diagnosis%20and%20treatment%20is%20low).

22. Xue MM, Glenn JS, Leung DH. Hepatitis D in Children. J Pediatr Gastroenterol Nutr. 2015 Sep;61(3):271–81.

23. UK Health Security Agency. Publication reference no. GOV-12265. 2022. UK Health security agency, briefing note. Screening tests for non Hep A-E cases of acute hepatitis (https://assets.publishing.service.gov.uk/goverment/uploads/system/uploads/attachment_data/file/1077027/acute-hepatitis-technical-briefing_3.pdf).

24. Ronan BA, Agrwal N, Carey EJ, De Petris G, Kusne S, Seville MT, et al. Fulminant hepatitis due to human adenovirus. Infection. 2014 Feb;42(1):105–11.

25. Naso MF, Tomkowicz B, Perry WL, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. BioDrugs. 2017 Aug;31(4):317–34.

26. Durmaz AA, Karaca E, Demkow U, Toruner G, Schoumans J, Cogulu O. Evolution of genetic techniques: past, present, and beyond. Biomed Res Int. 2015;2015:461524.

27. Cairns R, Brown JA, Wylie CE, Dawson AH, Isbister GK, Buckley NA. Paracetamol poisoning-related hospital admissions and deaths in Australia, 2004-2017. Med J Aust. 2019 Sep;211(5):218–23.

28. Nash E, Sabih AH, Chetwood J, Wood G, Pandya K, Yip T, et al. Drug-induced liver injury in Australia, 2009-2020: the increasing proportion of non-paracetamol cases linked with herbal and dietary supplements. Med J Aust. 2021 Sep 20;215(6):261–8.

29. Stormon MO, Hardikar W, Evans HM, Hodgkinson P. Paediatric liver transplantation in Australia and New Zealand: 1985-2018. J Paediatr Child Health. 2020 Nov;56(11):1739–46.

30. Meirelles Júnior RF, Salvalaggio P, Rezende MB de, Evangelista AS, Guardia B Della, Matielo CEL, et al. Liver transplantation: history, outcomes and perspectives. Einstein (Sao Paulo). 2015;13(1):149–52.

31. Kelly DA. Current results and evolving indications for liver transplantation in children. J Pediatr Gastroenterol Nutr. 1998 Aug;27(2):214–21.

32. McDiarmid S V. Management of the pediatric liver transplant patient. Liver Transpl. 2001 Nov;7(11 Suppl 1):S77-86.

33. Davis A, Rosenthal P, Glidden D. Pediatric liver retransplantation: Outcomes and a prognostic scoring tool. Liver Transplantation. 2009 Feb;15(2):199–207.

34. Tzakis AG, Gordon RD, Shaw BW, Iwatsuki S, Starzl TE. Clinical presentation of hepatic artery thrombosis after liver transplantation in the cyclosporine era. Transplantation. 1985 Dec;40(6):667–71.

35. Hoffer FA, Teele RL, Lillehei CW, Vacanti JP. Infected bilomas and hepatic artery thrombosis in infant recipients of liver transplants. Interventional radiology and medical therapy as an alternative to retransplantation. Radiology. 1988 Nov;169(2):435–8.

36. Zhang X, Zhang C, Huang H, Chen R, Lin Y, Chen L, et al. Primary nonfunction following liver transplantation: Learning of graft metabolites and building a predictive model. Clin Transl Med. 2021 Jul;11(7):e483.

37. Demetris AJ, Zeevi A, O'Leary JG. ABO-compatible liver allograft antibody-mediated rejection: an update. Curr Opin Organ Transplant. 2015 Jun;20(3):314–24.

38. Hübscher SG. Antibody-mediated rejection in the liver allograft. Curr Opin Organ Transplant. 2012 Jun;17(3):280–6.

39. Kim PTW, Demetris AJ, O'Leary JG. Prevention and treatment of liver allograft antibody-mediated rejection and the role of the "two-hit hypothesis". Curr Opin Organ Transplant. 2016 Apr;21(2):209–18.

40. O'Leary JG, Michelle Shiller S, Bellamy C, Nalesnik MA, Kaneku H, Jennings LW, et al. Acute liver allograft antibody-mediated rejection: an inter-institutional study of significant histopathological features. Liver Transpl. 2014 Oct;20(10):1244–55.

41. Choudhary NS, Saigal S, Bansal RK, Saraf N, Gautam D, Soin AS. Acute and Chronic Rejection After Liver Transplantation: What A Clinician Needs to Know. J Clin Exp Hepatol. 2017 Dec;7(4):358–66.

42. Choudhary NS, Saigal S, Bansal RK, Saraf N, Gautam D, Soin AS. Acute and Chronic Rejection After Liver Transplantation: What A Clinician Needs to Know. J Clin Exp Hepatol. 2017 Dec;7(4):358–66.

43. Mardare R, Hind J. Paediatric intestinal transplantation: where are we now? Paediatr Child Health. 2022 Nov;32(11):403–9.

44. Abu-El-Haija M, Kumar S, Quiros JA, Balakrishnan K, Barth B, Bitton S, et al. Management of Acute Pancreatitis in the Pediatric Population. J Pediatr Gastroenterol Nutr. 2018 Jan;66(1):159–76.

45. Wu D, Bampton TJ, Scott HS, Brown A, Kassahn K, Drogemuller C, et al. The clinical and genetic features of hereditary pancreatitis in South Australia. Medical Journal of Australia. 2022 Jun 20;216(11):578–82.

46. Muratore S, Freeman M, Beilman G. Total Pancreatectomy and Islet Auto Transplantation for Chronic Pancreatitis. Pancreapedia: Exocrine Pancreas Knowledge Base. 2015 Feb 20;

47. Stormon MO, Hardikar W, Evans HM, Hodgkinson P. Paediatric liver transplantation in Australia and New Zealand: 1985-2018. J Paediatr Child Health. 2020 Nov 10;56(11):1739–46.

48. 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. Learned Publishing. 2019 Jan 24;32(1):71–4.

49. Junger H, Knoppke B, Schurr L, Brennfleck FW, Grothues D, Melter M, et al. Good outcomes after repeated pediatric liver retransplantations: A justified procedure even in times of organ shortage. Pediatr Transplant. 2024 Mar 3;28(2).

50. Fan Y, Pan Q, Su H, Pu Z, Zhu L, Qi B, et al. Association between cerebral tissue oxygen saturation and neurodevelopmental delay in patients undergoing pediatric liver transplantation. 2023.

51. Squires RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006 May;148(5):652–8.

52. Matsukura H. Children with Acute Hepatitis of Unknown Cause. N Engl J Med. 2022 Nov 17;387(20):1906–7.

53. Hamdy A, Leonardi A. Superantigens and SARS-CoV-2. Pathogens. 2022 Mar 23;11(4).

54. Morfopoulou S, Buddle S, Torres Montaguth OE, Atkinson L, Guerra-Assunção JA, Moradi Marjaneh M, et al. Genomic investigations of unexplained acute hepatitis in children. Nature. 2023 May;617(7961):564–73.

55. Bhadri VA, Lee-Horn L, Shaw PJ. Safety and tolerability of cidofovir in high-risk pediatric patients. Transplant Infectious Disease. 2009 Aug 4;11(4):373–9.

56. Prerna Diksha. Immunosuppression-Responsive Paediatric Hepatitis And Acute Liver Failure With CD8+ T-Cell Rich Infiltrates . J Gastroenterol Hepatol. 2023 Sep 27;38(S2):235–45.

57. Gilbert MA, Loomes KM. Alagille syndrome and non-syndromic paucity of the intrahepatic bile ducts. Transl Gastroenterol Hepatol. 2021;6:22.

58. Meena BL, Khanna R, Bihari C, Rastogi A, Rawat D, Alam S. Bile duct paucity in childhood-spectrum, profile, and outcome. Eur J Pediatr. 2018 Aug;177(8):1261–9.

59. Cholico GN, Nault R, Zacharewski T. Cell-specific AHR-driven differential gene expression in the mouse liver cell following acute TCDD exposure. BMC Genomics. 2024 Aug 28;25(1):809.

60. NHS Blood and Transplant. NHS Pancreas Transplantation (https://www.nhsbt.nhs.uk/organ-transplantation/pancreas/). 2022.

61. Manay P, Turgeon N, Axelrod DA. Role of Whole Organ Pancreas Transplantation in the Day of Bioartificial and Artificial Pancreas. Curr Transplant Rep. 2020 Dec 27;7(4):223–9.

62. Callaway E. “It will change everything”: DeepMind’s AI makes gigantic leap in solving protein structures. Nature. 2020 Dec;588(7837):203–4.

63. Chavhan GB, Siddiqui I, Ingley KM, Gupta AA. Rare malignant liver tumors in children. Pediatr Radiol. 2019 Oct;49(11):1404–21.

64. Bhagat P, Vij M, Raju LP, Gowrishankar G, Menon J, Shanmugam N, et al. Update on the Pathology of Pediatric Liver Tumors: A Pictorial Review. Diagnostics. 2023 Nov 24;13(23):3524.

65. Raitio A, Losty PD. Treatment and outcomes in pediatric inflammatory myofibroblastic tumors – A systematic review of published studies. European Journal of Surgical Oncology. 2024 Jul;50(7):108388.

66. Dong Y, Zahid KR, Han Y, Hu P, Zhang D. Treatment of Pediatric Inflammatory Myofibroblastic Tumor: The Experience from China Children's Medical Center. Children (Basel). 2022 Feb 24;9(3).

67. Gröbner SN, Worst BC, Weischenfeldt J, Buchhalter I, Kleinheinz K, Rudneva VA, et al. The landscape of genomic alterations across childhood cancers. Nature. 2018 Mar 15;555(7696):321–7.

68. Alonso-Luna O, Mercado-Celis GE, Melendez-Zajgla J, Zapata-Tarres M, Mendoza-Caamal E. The genetic era of childhood cancer: Identification of high-risk patients and germline sequencing approaches. Ann Hum Genet. 2023 May 10;87(3):81–90.

69. Chan SH, Lim WK, Ishak NDB, Li ST, Goh WL, Tan GS, et al. Germline Mutations in Cancer Predisposition Genes are Frequent in Sporadic Sarcomas. Sci Rep. 2017 Sep 6;7(1):10660.

70. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Atomoxetine. [Updated 2021 Aug 25]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548671/.

71. Monge-Urrea F, Montijo-Barrios E. Drug-induced Liver Injury in Pediatrics. J Pediatr Gastroenterol Nutr. 2022 Oct 1;75(4):391–5.

72. Danan G, Teschke R. Drug-Induced Liver Injury: Why is the Roussel Uclaf Causality Assessment Method (RUCAM) Still Used 25 Years After Its Launch? Drug Saf. 2018 Aug 3;41(8):735–43.

73. Fu S, Wu D, Jiang W, Li J, Long J, Jia C, et al. Molecular Biomarkers in Drug-Induced Liver Injury: Challenges and Future Perspectives. Front Pharmacol. 2019;10:1667.

74. Zhang CJ, Meyer SR, O'Meara MJ, Huang S, Capeling MM, Ferrer-Torres D, et al. A human liver organoid screening platform for DILI risk prediction. J Hepatol. 2023 May;78(5):998–1006.

75. Molleston JP, Fontana RJ, Lopez MJ, Kleiner DE, Gu J, Chalasani N, et al. Characteristics of idiosyncratic drug-induced liver injury in children: results from the DILIN prospective study. J Pediatr Gastroenterol Nutr. 2011 Aug;53(2):182–9.

76. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology. 1989 Aug;97(2):439–45.

77. Li M, Luo Q, Tao Y, Sun X, Liu C. Pharmacotherapies for Drug-Induced Liver Injury: A Current Literature Review. Front Pharmacol. 2021;12:806249.

78. Wang Y, Wang Z, Gao M, Zhong H, Chen C, Yao Y, et al. Efficacy and safety of magnesium isoglycyrrhizinate injection in patients with acute drug-induced liver injury: A phase II trial. Liver International. 2019 Nov 21;39(11):2102–11.

79. Lieber CS. Role of S-adenosyl-L-methionine in the treatment of liver diseases. J Hepatol. 1999 Jun;30(6):1155–9.

80. Devarbhavi H, Aithal G, Treeprasertsuk S, Takikawa H, Mao Y, Shasthry SM, et al. Drug-induced liver injury: Asia Pacific Association of Study of Liver consensus guidelines. Hepatol Int. 2021 Apr;15(2):258–82.

81. Fontana RJ. Pathogenesis of Idiosyncratic Drug-Induced Liver Injury and Clinical Perspectives. Gastroenterology. 2014 Apr;146(4):914-928.e1.

82. Fontana RJ, Hayashi PH, Gu J, Reddy KR, Barnhart H, Watkins PB, et al. Idiosyncratic Drug-Induced Liver Injury Is Associated With Substantial Morbidity and Mortality Within 6 Months From Onset. Gastroenterology. 2014 Jul;147(1):96-108.e4.

83. Krishna Y, Schammel J, Schammel C, Schammel D, Young A, Trocha S. The great imposture: Eosinophilic Cholangitis. HPB. 2018 Sep;20:S686.

84. Rodgers MS, Allen JP, Koea JB, McCall JL. Eosinophilic cholangitis: a case of “malignant masquerade”. HPB (Oxford). 2001;3(3):235–9.

85. Matsumoto N, Yokoyama K, Nakai K, Yamamoto T, Otani T, Ogawa M, et al. A case of eosinophilic cholangitis: imaging findings of contrast-enhanced ultrasonography, cholangioscopy, and intraductal ultrasonography. World J Gastroenterol. 2007 Apr 7;13(13):1995–7.

86. Couper R. Future of paediatric gastroenterology. J Paediatr Child Health. 2020 Nov;56(11):1674–6.

87. Maccauro V, Fianchi F, Gasbarrini A, Ponziani FR. Gut Microbiota in Primary Sclerosing Cholangitis: From Prognostic Role to Therapeutic Implications. Digestive Diseases. 2024;42(4):369–79.

88. Spurkland A, Saarinen S, Boberg KM, Mitchell S, Broome U, Caballeria L, et al. HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. Tissue Antigens. 1999 May 5;53(5):459–69.

89. Miura F, Asano T, Amano H, Yoshida M, Toyota N, Wada K, et al. Resected case of eosinophilic cholangiopathy presenting with secondary sclerosing cholangitis. World J Gastroenterol. 2009 Mar 21;15(11):1394–7.

90. Kotlyar DS, Shum M, Hsieh J, Blonski W, Greenwald DA. Non-pulmonary allergic diseases and inflammatory bowel disease: a qualitative review. World J Gastroenterol. 2014 Aug 28;20(32):11023–32.

91. Rawla P, Samant H. Primary Sclerosing Cholangitis. 2024.

92. Kim YS, Hurley EH, Park Y, Ko S. Primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD): a condition exemplifying the crosstalk of the gut–liver axis. Exp Mol Med. 2023 Jul 18;55(7):1380–7.

93. Paediatric Primary Sclerosing Cholangitis-Ulcerative colitis. Audit of 2 year outcomes with oral Vancomycin treatment. J Pediatr Gastroenterol Nutr. 2021 May 2;72(S1):1–1313.

94. Allegretti JR, Kassam Z, Carrellas M, Mullish BH, Marchesi JR, Pechlivanis A, et al. Fecal Microbiota Transplantation in Patients With Primary Sclerosing Cholangitis: A Pilot Clinical Trial. Am J Gastroenterol. 2019 Jul;114(7):1071–9.

95. Shah A, Jones MP, Callaghan G, Fairlie T, Ma X, Culver EL, et al. Efficacy and safety of biologics in primary sclerosing cholangitis with inflammatory bowel disease: A systematic review and meta-analysis. Hepatol Commun. 2024 Jan;8(1).

96. Xiao A, Feng Y, Yu S, Xu C, Chen J, Wang T, et al. General anesthesia in children and long-term neurodevelopmental deficits: A systematic review. Front Mol Neurosci. 2022;15:972025.

97. Fragkos KC, Forbes A. Citrulline as a marker of intestinal function and absorption in clinical settings: A systematic review and meta-analysis. United European Gastroenterol J. 2018 Mar;6(2):181–91.

98. Varkey J. Graft assessment for acute rejection after intestinal transplantation: current status and future perspective. Scand J Gastroenterol. 2021 Jan 2;56(1):13–9.

99. Selvaggi G, Tzakis A. Current Status of Intestinal Transplantation. In: Regenerative Medicine Applications in Organ Transplantation. Elsevier; 2014. p. 481–92.

100. Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee. Tranfusion handbook; 8.5 Transfusion and organ transplantation (https://www.transfusionguidelines.org/transfusion-handbook/8-effective-transfusion-in-medical-patients/8-5-transfusion-and-organ-transplantation#:~:text=1%3A%20Renal%20transplantation,antigens%20on%20white%20blood%20cells). 2023.

101. Boyd SD, Stenard F, Lee DKK, Goodnough LT, Esquivel CO, Fontaine MJ. Alloimmunization to red blood cell antigens affects clinical outcomes in liver transplant patients. Liver Transpl. 2007 Dec;13(12):1654–61.

102. Hann A, Lembach H, Nutu A, Dassanayake B, Tillakaratne S, McKay SC, et al. Outcomes of normothermic machine perfusion of liver grafts in repeat liver transplantation (NAPLES initiative). Br J Surg. 2022 Mar 15;109(4):372–80.

103. Schlitt HJ, Barkmann A, Böker KH, Schmidt HH, Emmanouilidis N, Rosenau J, et al. Replacement of calcineurin inhibitors with mycophenolate mofetil in liver-transplant patients with renal dysfunction: a randomised controlled study. Lancet. 2001 Feb 24;357(9256):587–91.

104. Cholongitas E, Goulis I, Theocharidou E, Antoniadis N, Fouzas I, Imvrios G, et al. Everolimus with or without mycophenolate mofetil in a liver transplantation setting: a single-center experience. Ann Gastroenterol. 2018;31(5):613–20.

105. Pacheco MP, Carneiro-D'Albuquerque LA, Mazo DF. Current aspects of renal dysfunction after liver transplantation. World J Hepatol. 2022 Jan 27;14(1):45–61.

106. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013 Jul 25;499(7459):481–4.

107. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MMA, et al. Long-Term Culture of Genome-Stable Bipotent Stem Cells from Adult Human Liver. Cell. 2015 Jan;160(1–2):299–312.

108. Dianat N, Dubois-Pot-Schneider H, Steichen C, Desterke C, Leclerc P, Raveux A, et al. Generation of functional cholangiocyte-like cells from human pluripotent stem cells and HepaRG cells. Hepatology. 2014 Aug 20;60(2):700–14.

109. Akbari S, Arslan N, Senturk S, Erdal E. Next-Generation Liver Medicine Using Organoid Models. Front Cell Dev Biol. 2019;7:345.

110. Mun SJ, Ryu JS, Lee MO, Son YS, Oh SJ, Cho HS, et al. Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids. J Hepatol. 2019 Nov;71(5):970–85.

111. Hu H, Gehart H, Artegiani B, LÖpez-Iglesias C, Dekkers F, Basak O, et al. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell. 2018 Nov;175(6):1591-1606.e19.

112. Akbari S, Sevinç GG, Ersoy N, Basak O, Kaplan K, Sevinç K, et al. Robust, Long-Term Culture of Endoderm-Derived Hepatic Organoids for Disease Modeling. Stem Cell Reports. 2019 Oct;13(4):627–41.

113. Zabaleta N, Unzu C, Weber ND, Gonzalez-Aseguinolaza G. Gene therapy for liver diseases — progress and challenges. Nat Rev Gastroenterol Hepatol. 2023 May 16;20(5):288–305.

114. Wang B, Tang C, Lin E, Jia X, Xie G, Li P, et al. NIR-II fluorescence-guided liver cancer surgery by a small molecular HDAC6 targeting probe. EBioMedicine. 2023 Dec;98:104880.

115. Belghiti J, Sommacale D, Dondéro F, Zinzindohoué F, Sauvanet A, Durand F. Auxiliary liver transplantation for acute liver failure. HPB (Oxford). 2004;6(2):83–7.

116. Figiel W, Smoter P, Krasnodębski M, Rykowski P, Morawski M, Grąt M, et al. Predictors of Long-Term Outcomes After Liver Transplantation Depending on the Length of Cold Ischemia Time. Transplant Proc. 2022 May;54(4):1025–8.

117. Tingle SJ, Dobbins JJ, Thompson ER, Figueiredo RS, Mahendran B, Pandanaboyana S, et al. Machine perfusion in liver transplantation. Cochrane Database of Systematic Reviews. 2023 Sep 12;2023(9).

118. Sorensen LG, Neighbors K, Martz K, Zelko F, Bucuvalas JC, Alonso EM. Cognitive and Academic Outcomes after Pediatric Liver Transplantation: Functional Outcomes Group (FOG) Results. American Journal of Transplantation. 2011 Feb;11(2):303–11.

119. Woolfson JP, Perez M, Chavhan GB, Johara FT, Lurz E, Kamath BM, et al. Sarcopenia in Children With End-Stage Liver Disease on the Transplant Waiting List. Liver Transplantation. 2021 May 27;27(5):641–51.

120. Veraldi S, Pietrobattista A, Soglia G, Monti L, Alterio T, Mosca A, et al. Sarcopenia in children with chronic liver disease: Prevalence and impact on liver transplant outcomes. Front Pediatr. 2022 Oct 18;10.

121. Hager A, Boule N, Pritchard L, Hodgetts S, Noga M, Guo Y, et al. Sarcopenia in Children Post Liver Transplant: Development of a Home-Based Video Program to Support Muscle Strength and Function—A Pre–Post Controlled Pilot Study. Clin Transplant. 2024 Sep 10;38(9).

122. Feldman AG, Whitington PF. Neonatal hemochromatosis. J Clin Exp Hepatol. 2013 Dec;3(4):313–20.

123. Rand EB, Karpen SJ, Kelly S, Mack CL, Malatack JJ, Sokol RJ, et al. Treatment of neonatal hemochromatosis with exchange transfusion and intravenous immunoglobulin. J Pediatr. 2009 Oct;155(4):566–71.

124. Williamson SL, Rasanayagam CN, Glover KJ, Baptista J, Naik S, Satodia P, et al. Rapid exome sequencing: revolutionises the management of acutely unwell neonates. Eur J Pediatr. 2021 Dec;180(12):3587–91.

125. Chinsky JM, Singh R, Ficicioglu C, van Karnebeek CDM, Grompe M, Mitchell G, et al. Diagnosis and treatment of tyrosinemia type I: a US and Canadian consensus group review and recommendations. Genetics in Medicine. 2017 Dec;19(12):1380–95.

126. Park JH, Marquardt T. Treatment Options in Congenital Disorders of Glycosylation. Front Genet. 2021;12:735348.

127. Khazaaleh S, Babar S, Alomari M, Imam Z, Chadalavada P, Gonzalez AJ, et al. Outcomes of total pancreatectomy with islet autotransplantation: A systematic review and meta-analysis. World J Transplant. 2023 Jan 18;13(1):10–24.

128. Ahmad SA, Lowy AM, Wray CJ, D'Alessio D, Choe KA, James LE, et al. Factors associated with insulin and narcotic independence after islet autotransplantation in patients with severe chronic pancreatitis. J Am Coll Surg. 2005 Nov;201(5):680–7.

129. Ramzy A, Thompson DM, Ward-Hartstonge KA, Ivison S, Cook L, Garcia R V, et al. Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes. Cell Stem Cell. 2021 Dec 2;28(12):2047-2061.e5.

130. Tangamornsuksan W, Lohitnavy O, Kongkaew C, Chaiyakunapruk N, Reisfeld B, Scholfield NC, et al. Association of HLA-B*5701 Genotypes and Abacavir-Induced Hypersensitivity Reaction: A Systematic Review and Meta-Analysis. Journal of Pharmacy & Pharmaceutical Sciences. 2015 Feb 15;18(1):68.

131. Wu R, Cheng YJ, Zhu LL, Yu L, Zhao XK, Jia M, et al. Impact of HLA-B*58:01 allele and allopurinol-induced cutaneous adverse drug reactions: evidence from 21 pharmacogenetic studies. Oncotarget. 2016 Dec 6;7(49):81870–9.

132. McCormack M, Alfirevic A, Bourgeois S, Farrell JJ, Kasperavičiūtė D, Carrington M, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. N Engl J Med. 2011 Mar 24;364(12):1134–43.

133. Chen WT, Wang CW, Lu CW, Chen CB, Lee HE, Hung SI, et al. The Function of HLA-B*13:01 Involved in the Pathomechanism of Dapsone-Induced Severe Cutaneous Adverse Reactions. J Invest Dermatol. 2018 Jul;138(7):1546–54.

134. Nicoletti P, Aithal GP, Chamberlain TC, Coulthard S, Alshabeeb M, Grove JI, et al. Drug-Induced Liver Injury due to Flucloxacillin: Relevance of Multiple Human Leukocyte Antigen Alleles. Clin Pharmacol Ther. 2019 Jul 19;106(1):245–53.

135. Cornejo Castro EM, Carr DF, Jorgensen AL, Alfirevic A, Pirmohamed M. HLA-allelotype associations with nevirapine-induced hypersensitivity reactions and hepatotoxicity: a systematic review of the literature and meta-analysis. Pharmacogenet Genomics. 2015 Apr;25(4):186–98.

136. Nicoletti P, Aithal GP, Bjornsson ES, Andrade RJ, Sawle A, Arrese M, et al. Association of Liver Injury From Specific Drugs, or Groups of Drugs, With Polymorphisms in HLA and Other Genes in a Genome-Wide Association Study. Gastroenterology. 2017 Apr;152(5):1078–89.

137. Bonkovsky HL, Ghabril M, Nicoletti P, Dellinger A, Fontana RJ, Barnhart H, et al. Drug-induced liver injury (DILI) ascribed to non-steroidal anti-inflammatory drugs (NSAIDs) in the USA-Update with genetic correlations. Liver Int. 2024 Jun;44(6):1409–21.

138. Hautekeete ML, Horsmans Y, Van Waeyenberge C, Demanet C, Henrion J, Verbist L, et al. HLA association of amoxicillin-clavulanate--induced hepatitis. Gastroenterology. 1999 Nov;117(5):1181–6.

139. Daly AK. Genetics of drug-induced liver injury: Current knowledge and future prospects. Clin Transl Sci. 2023 Jan 4;16(1):37–42.

140. Pillaye JN, Marakalala MJ, Khumalo N, Spearman W, Ndlovu H. Mechanistic insights into antiretroviral drug-induced liver injury. Pharmacol Res Perspect. 2020 Aug 8;8(4).

141. Nicoletti P, Devarbhavi H, Goel A, Venkatesan R, Eapen CE, Grove JI, et al. Genetic Risk Factors in Drug-Induced Liver Injury Due to Isoniazid-Containing Antituberculosis Drug Regimens. Clin Pharmacol Ther. 2021 Apr 5;109(4):1125–35.

142. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. 2017.

143. Nicoletti P, Dellinger A, Li YJ, Barnhart H, Phillips E, Chalasani N, et al. HLA-B*53:01 Is a Significant Risk Factor of Liver Injury due to Phenytoin and Other Antiepileptic Drugs in African Americans. Am J Gastroenterol. 2024 Jan 1;119(1):200–2.