MELIOIDOSIS:

EPIDEMIOLOGY, PATHOPHYSIOLOGY AND MANAGEMENT

Presented By

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DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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"…Shall I demonstrate your own ignorance? What do you know, pray, of Tapanuli fever? What do you know of the black Formosa corruption?"

"I have never heard of either."

"There are many problems of disease, many strange pathological possibilities, in the East, Watson."

The Dying Detective, Sir Arthur Conan Doyle

SUMMARY

In under a century, melioidosis, the infection due to *Burkholderia pseudomallei,* has emerged from Whitmore's series of glanders-like infections amongst the morphia addicts in Burma to a major cause of mortality in northeastern Thailand and northern Australia. Also endemic in other parts of south-east Asia, melioidosis may have varied presentations ranging from severe, overwhelming infection to chronic, low grade disease.

Observational evidence had suggested that granulocyte colony stimulating factor (G-CSF), a naturally occurring substance produced by the body in response to infection, may have been useful in reducing the high mortality associated with the more severe forms of this infection. Other observations linked the occurrence of this disease to various environmental factors, such as contamination of drinking water and the annual rainfall. This thesis explores and attempts to quantify these associations.

There are three parts to this thesis. In the first part, I reviewed the epidemiology and management of patients with melioidosis. The use of G-CSF and meropenem was associated with a fall in mortality, although other factors may have at least partially contributed to this effect.

In the second part, I progressed towards a clinical trial of G-CSF. There was no other evidence supporting the use of G-CSF in severe sepsis and ethical issues precluded a trial in Darwin. There was not evidence from laboratory models of G-CSF action in melioidosis to support the use of G-CSF in patients, although there remained some doubt regarding the applicability of such models to human disease. I examined clinical methods to identify patients at high risk of death from melioidosis. A simple scoring system based on clinical and laboratory parameters was developed and externally validated. However, clinical definitions of severe sepsis appeared to be better predictors of mortality. A clinical trial based on clinical definitions was commenced in Thailand.

In the final part, I explored the question of whether different strains or *B. pseudomallei* or different environmental conditions caused different patterns of infection. There was no evidence that strain types of this bacterium determine the pattern or severity of disease, but weather conditions appeared to influence the distribution of disease in northern Australia.

PUBLICATIONS ARISING OUT OF THIS WORK

Published journal articles

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Unpublished manuscripts

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34. **Cheng AC**, Chierakul W, Currie BJ, Day N, Chaowagul W, White NJ. Validation of a prognostic scoring system for melioidosis. (In preparation)

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DECLARATION OF THE AUTHOR'S CONTRIBUTION

This thesis is substantially my own work and was implemented under the supervision of Bart Currie and Nick Anstey. I wrote all chapters and manuscripts and analyzed all data.

I acknowledge the contributions to this work made by others:

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- The following people provided data used in the 2001/2 melioidosis report: Jeffrey Hanna (Tropical Public Heath Unit, Queensland Health, Cairns, Queensland), Robert Norton (Department of Microbiology, The Townsville Hospital, Queensland Health Pathology & Scientific Services, Townsville, Queensland), Susan Hills (Tropical Public Heath Unit, Queensland Health, Townsville, Queensland), Josh Davis (Royal Darwin Hospital), Vicki Krause (Disease Control, NT Department of Health and Community Services, Darwin, NT), Gary Dowse (Communicable Diseases Control Branch, Department of Health, Western Australia), Tim Inglis (PathCentre, WA Department of Health, Perth, Western Australia). Jeffrey Hanna wrote the discussion regarding the Queensland cases.
- In the chapters on the epidemiology of melioidosis, Mr Mark Mayo and Mr Daniel Gal are involved in ongoing water sampling and performance of PFGE strain typing to define case-clustering. I analyzed the data with the help of Daniel Gal who entered gel images into the Bionumerics program.
- Ms Kathryn Clarkson, Mr Daryl Day, Mr Frank Richards and Mr Andrew Mills at the Power and Water Corporation (Darwin, NT) provided water supply data and Mr Peter Bate from the Bureau of Meteorology, Darwin provided rainfall data.
- Dr Nick Day suggested the method of analysis for PFGE data using the similarity matrix and Simpson's index of diversity, previously used in a thesis by Dr Caterin Moore (D.Phil, Oxford).

- MLST was performed at Imperial College London by Daniel Godoy under the supervision of Prof Brian Spratt. Prof Spratt helped with the analysis and provided significant editorial comment on the draft manuscript.

1. Declaration of potential conflicting interests

During the course of this research, we received an unrestricted donation of lenograstim (Granocyte, Chugai Pharmaceuticals, Japan) from Merck Australia, who at the time owned Faulding Pharmaceuticals, the Australian distributor of lenograstim. This comprised 50 ampoules of G-CSF valued at approximately A$6000. This donation was used to perform G-CSF replacement studies in G-CSF knockout mice and, in part, the clinical trial in Thailand. The company did not seek, and nor did we offer, a review of our research as a condition of this donation.

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Abbreviations

µg	Micrograms
129/OLA	Inbred mouse strain 129, substrain OLA
xº y' W	x degrees, y minutes west of Greenwich meridian
xº y' S	x degrees, y minutes south of the equator
ºC	degrees Celsius
95% CI	95% confidence interval
A$	Australian dollars
ACCP/SCCM	American College of Chest Physicians/Society of Critical Care Medicine
AF	Atrial fibrillation
AMI	Acute myocardial infaction
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APACHE	acute physiology and chronic health evaluation score
Ara^+	Arabinose assimilating
ARDS	Acute respiratory distress syndrome
ARF	Acute renal failure
AZT	Azidothymidine; zidovudine
B. pseudomallei	*Burkholderia pseudomallei*
Balb/c	Inbred mouse strain Balb/c
BKA	Below knee amputation
C57B6	Inbred mouse strain C57 black, substrain 6
CD-ROM	Compact disc read only memory
cfu	Colony forming units
CNS	Central nervous system
COAD	Chronic obstructive airways disease
CVVHF	Continuous veno-venous haemofiltration
D	Simpson's index of diversity
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
ECMO	Extra-corporeal membrane oxygenation
EMBASE	*Excerpta Medica* database

GAM	Generalized additive model
G-CSF	Granulocyte colony stimulating factor
G-CSF -/-	Homozygous G-CSF gene knockout mice
GI bleed	Gastrointestinal tract bleeding
GIS	Geographical information systems
HBA	Horse blood agar
HBV	Hepatitis B
HEPA	High efficiency particulate air filtration
HIV	Human immunodeficiency virus
HTLV-1	Human T-cell leukaemia virus
ICU	Intensive Care Unit
IFN	Interferon
Ig	Immunoglobulin
IHD	Ischaemic heart disease
IL	Interleukin
IV	Intravenous
LB	Luria Bertani
LD_{50}	Lethal dose for 50%
LOD	Logistic Organ Dysfunction
Log	Logarithm (base 10 unless specified)
LR^+	Positive likelihood ratio
MEDLINE	Medical Literature Analysis and Retrieval System Online
MIC	Minimum inhibitory concentration
mL	Milliliters
MLEE	Multi-locus enzyme electrophoresis
MLST	Multi-locus sequence testing
mmol	Millimoles
MODS	Multiple Organ Dysfunction Score
MRSA	Methicillin-resistant *Staphylococcus aureus*
n=	Number in sample
ng	Nanograms
nm	Nanometers
NT	Northern Territory, Australia
NZ	New Zealand

p=	Probability equals
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
pH	Power of hydrogen ion concentration
PT	PFGE strain type
Qld	Queensland, Australia
RAPD	Randomly amplified polymorphic DNA
RCT	Randomized controlled trial
RDH	Royal Darwin Hospital
ROC	Receiver operator characteristic
S. pyogenes	*Streptococcus pyogenes*
SCC	Squamous cell carcinoma
SOFA	Sequential Organ Failure Assessment
SpeI	Restriction enzyme *SpeI*
ThB, ฿	Thai baht (A$1≈ThB23-26 at the time of writing)
™	Trademark
TMP-SMX	Trimethoprim-sulphamethoxazole (cotrimoxazole)
TNF	Tumor necrosis factor
t-test	Student's t-test
UPGMA	Unweighted pair group matching band average
US$	United States dollars (A$1≈US$0.60-0.65 at the time of writing)
WA	Western Australia
WCC	White cell count
yr	Year
Σ	Sum of
σ^2	Variance
χ^2	Chi-squared statistic