Digging for melioidosis

New insights into the epidemiology and pathophysiology

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CHAPTER 12

SUMMARY
Melioidosis is an often fatal infectious disease caused by the Gram-negative soil dwelling bacterium *Burkholderia pseudomallei*, and an important cause of community-acquired pneumonia and sepsis in Southeast Asia and Northern Australia.\(^1\)\(^2\) Globally, melioidosis accounts for 169,000 cases and 89,000 estimated deaths per year, yet the true burden remains poorly understood.\(^1\) Diagnosis requires specific microbiology facilities and experienced microbiologists are needed for the isolation and identification of *B. pseudomallei*.\(^1\) *B. pseudomallei* is considered a potential bioterror threat weapon due to its high case-fatality, intrinsic resistance against several key antibiotics, and potential transmission via respiratory aerosols.\(^4\)

The central aims of this thesis are to provide new insights into the burden of melioidosis, the distribution of *B. pseudomallei* and *B. thailandensis* in sub-Saharan Africa, the pathophysiology of melioidosis, and to open up avenues for new treatment strategies and vaccines.

**Chapter 1** provides an introduction on the known epidemiology and pathophysiology of *B. pseudomallei* infection and a rationale for the relevance of a global burden of disease study on melioidosis for health policy makers. We introduce the distribution of melioidosis and its causative agent *B. pseudomallei*, associated disease manifestations, risk factors, the virulence factors of the bacterium, and the host pathogen interactions.

**Section I - Burden of Melioidosis**

The global importance of melioidosis is substantial, yet the true burden remains to be elucidated. Melioidosis, however, is not recognized as an official Neglect Tropical Disease (NTD) by the World Health Organization (WHO) even though it has high mortality rates and is potentially preventable and treatable.\(^1\) Accordingly, Section I focusses on the burden of melioidosis. **Chapter 2** quantifies the global disability-adjusted life years (DALYs) of melioidosis at country, regional, and global levels and aims to inform health officials and policy makers on the potential size of the problem. The DALY is a metric that can be used to summarize morbidity, disability, and mortality into a single index. Using a broad search strategy, with no language restrictions, all human culture-confirmed cases between 1990 and 2015 were systematically reviewed. An incidence based disease model was constructed and quantitative data were retained from 475 studies on mortality, sex, infectious and post-infectious sequelae, antibiotic treatment and symptom duration. All this was combined with recognized disabil-
ity weights (DWs) and expert panel discussions. Pneumonia and sepsis/septic shock were among the most common disease outcomes, occurring in 65.8% of the patients. Incidence and mortality from melioidosis were about twice as high for men than in women. Predicted global incidence and mortality rates were integrated into the disease model to estimate the worldwide melioidosis DALYs by age, sex, country and region. For the year 2015, the total DALYs was estimated to be 4.6 million (UI 3.2-6.6) or 84 per 100,000 people (UI 58-120). Years of live lost (YLLs) contributed to 98.9% (UI 97.7-99.5) of the total DALYs. Known risk factors were present in the vast majority of patients, most notably diabetes, chronic liver disease or alcohol abuse, chronic kidney disease, and chronic lung disease. Putting this into context, the estimated global burden of melioidosis in terms of DALYs is higher than recognized NTDs, such as leptospirosis, dengue, and leishmaniasis.

With increasing numbers of immune-compromised travelers, Chapter 3 provides a systematic overview of all imported human melioidosis cases in a non-endemic country, the Netherlands, for over a 25-year period. In collaboration with the Dutch National Institute for Public Health and the Environment, we systematically reviewed all *B. pseudomallei* positive culture results in Dutch medical microbiology laboratories. Microbiology laboratories response rate was 100%. In total, 33 returned Dutch travelers with melioidosis were identified (70% male; median age 54 years (range 21-83)). Risk factors were present in most patients (n=23, 70%), most notably diabetes (n=8, 24%) and cystic fibrosis (n=3, 9%). Infections were acquired in amongst others Thailand, Brazil, Indonesia, Panama, and The Gambia. Foci of infections included amongst others pneumonia, genitourinary, skin-soft tissue, lymphadenitis, central nervous system, thyroid gland, mycotic aneurysm, and otitis externa. Twelve (36%) patients developed sepsis and/or septic shock. Relapse occurred in 15 (5%) and mortality in four (12%) patients. Post-mortem analysis showed extensive metastatic (micro)abscesses in amongst others the liver, spleen, adrenal gland, and bone marrow. This largest series of travelers with melioidosis to date, also shows that imported cases can serve as sentinels for non-endemic areas and inform pre-travel advice and post-travel clinical management.

**Section II - In Search of Melioidosis in Africa**

We hypothesized that melioidosis is present in Africa, but that the disease is underdiagnosed because of a lack of diagnostic facilities and awareness
amongst clinicians. In Section II, we focus on the distribution of \( B. \) \textit{pseudomallei} and \( B. \) \textit{thailandensis} in parts of Western Africa. A modelling study\textsuperscript{3} estimated that \( B. \) \textit{pseudomallei} is widely present in the soil in sub-Saharan Africa and many patients may die unidentified as suffering from melioidosis. Chapter 4 provides an overview of all \( B. \) \textit{pseudomallei} literature reports in humans, animals, as well as the environment in Africa showing that reported cases in Africa are few and isolated. This is possibly due to under recognition and under reporting, and may represent ‘the tip of the iceberg’.\textsuperscript{1} The clinical spectrum of the 13 reported human cases in Africa corresponds with the clinical picture observed in other endemic regions such as Southeast Asia and Northern Australia. Febrile diseases can sometimes mistakenly be credited to malaria, possibly masking the presence of melioidosis. Soil sampling studies and improvements in diagnostic facilities could provide a more accurate and comprehensive map of the environmental distribution and burden of \( B. \) \textit{pseudomallei} across the African continent. Chapter 5 describes a first fully translational melioidosis research project in the Central African country of Gabon. A first serology study showed positive antibody titters (1≥40) in 5 out of 304 (1.6%) healthy school age children. Our environmental surveillance study demonstrated the presence of \( B. \) \textit{pseudomallei} in three out of eight sites. \( B. \) \textit{pseudomallei} was isolated in 3% out of 800 soil samples. A prospective fever cohort study detected the first melioidosis patient in Gabon. A 62-year old Gabonese woman with an abscess on her leg and eventually died because of septic shock. Her blood culture was positive for \( B. \) \textit{pseudomallei}. Using molecular sequence typing (MLST) and whole genome sequencing (WGS), we identified a novel \( B. \) \textit{pseudomallei} sequence type. Additionally, we were the first to describe the presence of \( B. \) \textit{thailandensis} in the environment of Central Africa.

Chapter 6 characterizes the inflammatory response induced by the \( B. \) \textit{pseudomallei} isolate, derived from the fatal Gabonese melioidosis case, using our experimental mouse model. We compared this isolate with the Thai reference strains \( B. \) \textit{pseudomallei} 1026b and \( B. \) \textit{thailandensis} E-264. Ex \textit{vivo}, no differences were observed in terms of pro-inflammatory cytokine release upon stimulation of whole blood, peritoneal macrophages and, alveolar macrophages. In \textit{vivo}, however, mice showed reduced bacterial loads in lung, liver, and blood upon infection with the Gabonese \( B. \) \textit{pseudomallei} strain when compared to the reference strain. Remarkably, the \textit{in vivo} virulence in terms of bacterial counts of the Gabonese strain was similar to the considered avirulent \( B. \) \textit{thailandensis} strain. To find a potential
cause of the decreased virulence of the Gabonese isolate we made use of the Basic Local Alignment Search Toll (BLAST) algorithm. Genomic comparison between strains showed differences in regions containing a fimbriae/adhesion virulence protein (more specifically the type IV fimbrial biogenesis protein PilY1).

Modelling studies have predicted that hundreds of patients may suffer from melioidosis in the Western African country of Sierra Leone. In the medical literature, however, only one report of a case of melioidosis in Sierra Leone has been published. The less virulent \textit{B. thailandensis} is genetically closely related to \textit{B. pseudomallei} and is also known to be able to cause disease in humans. Therefore, Chapter 7 tries to determine the presence of \textit{B. pseudomallei} and \textit{B. thailandensis} in the environment of Central Sierra Leone. Environmental surveillance across 10 sites was conducted in central Sierra Leone. Culture of 1,000 soil samples isolated 32 \textit{Burkholderia} strains from 25 soil samples. All isolates were identified as \textit{B. thailandensis} with MLST and 16S rRNA sequence analyses. Seven novel \textit{B. thailandensis} sequence types (STs) were described. However, the presence of \textit{B. pseudomallei} in the environment could not be established.

Section III - Pathogenesis of Melioidosis and Development of a Novel Vaccine

In Section III, we shift our focus to the pathogenesis of melioidosis and the development of a potential novel vaccine. Chapter 8 describes the role of platelets in the host response against \textit{B. pseudomallei} infection, as these cells are increasingly being recognized as key players in inflammation and immunity. Admission platelet counts were determined in 1,160 Thai culture-proven melioidosis patients. Thrombocytopenia was present in 31% of melioidosis patients and predicted mortality even after adjustment for important confounders, such as age and sex. During experimental murine melioidosis, platelet counts were also reduced, mimicking the clinical scenario. \textit{B. pseudomallei} infected mice treated with a high or low dose platelet depleted antibody showed increased mortality and induced bacterial loads in the lung and liver, as compared to mice with normal platelet counts. Low platelet counts had a modest effect on early neutrophil influx in the lung, but no impact on neutrophil extracellular trap formation (NETs). Similar to their role in hemostasis, platelet depletion impaired vascular integrity and induced early lung bleeding. Additional studies using Glycoprotein Ib-\alpha-deficient mice (GPIb\alpha), which lack important platelet receptor GPIb\alpha, were conducted. \textit{B. pseudomallei} infected GPI-
bα mice had reduced platelet counts together with an impaired local host defense in the lung, indicating that GPIbα may play a role in the protective effect of platelets. Taken together, thrombocytopenia is an independent predictor of mortality in melioidosis patients and, during experimental melioidosis, platelets play a protective role in both innate immunity and vascular integrity.

Chapter 9 studies the changes in Von Willebrand factor (vWF) levels in patients with melioidosis. The vWF protein is involved in hemostasis and platelet aggregation. Since endothelial stimulation by endotoxin, pro-inflammatory cytokines, and thrombin all occur during melioidosis, we hypothesized that these would result in derangements of vWF. In line with the findings from Chapter 8, platelet counts were significantly lower in Thai melioidosis patients compared to control patient and correlated with mortality. In addition, our studies showed that vWF antigen levels were higher in melioidosis patients compared to controls. vWF propeptide levels were increased in melioidosis patients suggesting increased endothelial stimulation. ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type-1 motif member and known as the main regulator of vWF activity by cleavage, was also reduced probably because of decreased clearance. vWF levels, however, did not correlate with platelet counts, suggesting that thrombocytopenia in melioidosis has other main drivers.

The gut microbiota is increasingly being recognized as a key mediator in the host defense against bacterial infections. Chapter 10 examines the composition and function of the intestinal microbiota during experimental murine melioidosis. Mice infected with B. pseudomallei showed a clear change in fecal bacterial composition with strongly increased Proteobacteria and decreased Actinobacteria. Subsequently, B. pseudomallei infected mice were treated with a broad spectrum of antibiotics to disrupt the microbiota. Microbiota depleted mice showed increased early bacterial dissemination and systemic inflammation compared to control mice after intranasal infection with B. pseudomallei. Antibiotic induced depletion of the gut microbiome only has a modest effect on survival and organ pathology in this lethal model of experimental melioidosis. Strikingly, alveolar macrophages derived from microbiota disrupted mice showed a decreased capacity to phagocytose B. pseudomallei. Taken together, these data identify the gut microbiota as a potential modulator of innate immunity during B. pseudomallei infection.

Currently, there is no licensed vaccine for the prevention of melioido-
sis. Moreover, there is need for a rapidly working vaccine against melioidosis as \textit{B. pseudomallei} is being considered as a potential bioterror weapon. Therefore, in \textbf{Chapter 11} we investigate the use of a new plasmid DNA vaccine against \textit{B. pseudomallei}. Flagellin is a suitable antigen for vaccine developments as it is seen as an important virulence factor of \textit{B. pseudomallei}.\textsuperscript{22} We tested several flagellin DNA vaccines in our experimental mouse model for melioidosis using a skin tattoo method. A vaccine encoding a cellular secretion flagellin proved to be most effective in protection against melioidosis using tattooing with a 10-fold reduction in bacterial loads in the lungs and distant organs. Strikingly, a single dose of the same flagellin DNA intranasally administrated, led to greater than thousand fold lower bacterial loads and improved survival as compared to control mice.
REFERENCES