

## SECTION B: BACKGROUND, TOOLS AND MODELS FOR A CLINICAL TRIAL

Current data supporting the use of G-CSF has been reviewed in chapter 3. In this section, background data and clinical tools were developed and evaluated, with the aim of undertaking a clinical trial of G-CSF in the treatment of melioidosis.

In chapter 5, a systematic review of both published and unpublished evidence examining the use of G-CSF in pneumonia and sepsis was made. These studies suggested that G-CSF was not associated with clinical benefits, but equally did not appear to worsen sepsis-related organ dysfunction. In all studies, enrolment criteria resulted in significant delays in administration of G-CSF that may have contributed to their negative results. The protocol and review of this study was published in the *Cochrane Database of Systematic Reviews* [1].

A review of the ethical issues involved in a clinical trial was performed in chapter 6. It was concluded that our previous evidence of efficacy, however confounded, resulted in a disturbance in “patient equipoise”, the state of uncertainty regarding the potential risks and benefits associated with the trial alternatives. No satisfactory alternatives could be found to allow the performance of this trial in Australia, but it was concluded that a trial could be ethically conducted in Thailand. This was published in the *British Medical Journal* [2].

In chapter 7, laboratory models of G-CSF action was explored. It could not be demonstrated that G-CSF augmented the bactericidal activity of whole blood in an *in vitro* assay. This was published in the *European Journal of Clinical Microbiology and Infectious Diseases* [3].

Clinical tools to identify patients at a high risk of death were developed and evaluated in chapters 8, 9 and 10. In chapter 8, it was found that C-reactive protein levels were elevated in patients that ultimately died, but patients were better identified by clinical predictors of severity. This was reported in the *American Journal of Tropical Medicine and Hygiene* [4]. Similarly, a simple scoring system

was developed in chapter 9 based on markers of organ dysfunction. However, on evaluation in a Thai population, clinical markers of severity were equally predictive of mortality. The development of this scoring system was published in the *Transactions of Tropical Medicine and Hygiene* [5]; the validation of this tool has not been submitted for publication as yet.

Finally, progress toward a clinical trial of G-CSF in Thailand is detailed in chapter 11, together with the lessons learned during the conduct of this trial. The protocol is detailed as an appendix.

### References

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## **5. A systematic review of G-CSF as an adjunct to antibiotics in the treatment of pneumonia in adults**

### **5.1. Introduction**

This review explores the use of granulocyte-colony stimulating factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in non-neutropenic adults. Much of the mortality associated with pneumonia is related to sepsis, thought to represent a state of uncontrolled inflammation. Immunomodulation of this response, using physiological doses of corticosteroids [1] and recombinant activated protein C [2] have been associated with improved outcomes. G-CSF, a naturally occurring cytokine, may augment the neutrophil response to bacterial infections.

Recombinant G-CSF has been shown to increase proliferation and differentiation of neutrophil precursors. It has found widespread use in reducing the duration of febrile neutropenia following cytotoxic chemotherapy [3], although its effect on mortality is questionable [4]. Other accepted indications include neutropenia following bone marrow transplantation, the mobilization of peripheral blood progenitor cells in healthy donors and in the treatment of severe congenital neutropenia.

Its use in non-neutropenic infection is based on three possible mechanisms of action:

- Enhanced chemotaxis, superoxide production and killing activity [5];
- Immunomodulation of the cytokine response in sepsis [6];
- A possible increase in intracellular uptake of antibiotics [7].

However, a possible concern with its use in sepsis is the role of neutrophils in the development of organ dysfunction, and in particular acute respiratory distress syndrome (ARDS). Increasing neutrophil number and function may increase the risk of these adverse sequelae. A review of 84 cases of probable G-CSF related pulmonary toxicity, mostly administered following chemotherapy, found that G-CSF might increase the toxicity associated with cytotoxic agents and infectious insults in neutropenic patients [8].

G-CSF has shown promise in the treatment of infection in non-neutropenic hosts in many animal models [9, 10]. It should be distinguished from GM-CSF (sargramostim), which is used occasionally in stem cell mobilization and to promote engraftment following bone marrow transplantation.

Community-acquired pneumonia is the leading cause of death from infectious disease and results in approximately 600,000 admissions per year in the United States [11]. Both community-acquired pneumonia and hospital-acquired pneumonia are associated with a significant mortality [12, 13]. A number of trials of G-CSF in the treatment of pneumonia have been performed. It was felt that a review and meta-analysis of these trials would provide vital background information in clarifying the role of this immunomodulatory therapy in melioidosis and aid design of possible future trials.

In this study, the effectiveness and safety of G-CSF as an adjunct to antibiotics for the treatment of pneumonia in non-neutropenic adults were assessed.

## **5.2. Methods**

### **5.2.1. Inclusion criteria**

Only randomized controlled trials were included in this review.

The population studied included hospitalized adult patients (older than 18 years) with:

1. Community-acquired pneumonia;
2. Hospital-acquired pneumonia, including ventilator-associated pneumonia.

"Community-acquired pneumonia" was defined as follows:

- Clinical features suggestive of lower respiratory tract infection (such as fever, cough, pleuritic chest pain, examination suggestive of consolidation);
- Chest x-ray demonstrating new infiltrate suggesting pneumonia;
- Onset in community setting (outpatient or less than 48 hours following admission to hospital);
- No alternative diagnosis at admission or during follow-up.

There has been controversy regarding the optimal definition of hospital-acquired pneumonia [14] and a paucity of studies of mortality. In general, hospital acquired pneumonia is associated with a high mortality [12]. In this review, "hospital acquired pneumonia" was regarded as a clinical definition [15]:

- Onset of illness more than 72 hours following admission;
- New infiltrate on chest x-ray;
- Signs of sepsis (leucocytosis, fever, tachypnoea, tachycardia);
- Increasing sputum production;
- No alternative diagnosis (such as Acute Respiratory Distress Syndrome [ARDS]) at time of evaluation or follow-up.

"Suspected ventilator associated pneumonia", a sub-set of patients with hospital-acquired pneumonia, was defined as:

- Hospital acquired pneumonia (defined above);
- Patients intubated for more than 72 hours.

"Confirmed ventilator associated pneumonia" was defined as 1 and 2 above, supported by quantitative microbiological techniques [16], including:

- Protected specimen brush sampling with quantitative culture techniques;
- Quantitative cultures of bronchoalveolar lavage;
- Quantitative cultures of endotracheal aspirates.

It was originally intended to only include patients with severe community acquired pneumonia, but subsequently found that the studies identified were performed prior to the development of standard definitions of severity [13, 17, 18]. A group with "severe sepsis" was incorporated and defined by standard criteria [19] which detail definitions of sepsis (fever, tachycardia, tachypnoea and leucocytosis) and end-organ perfusion abnormalities (hypotension, oliguria, gas exchange abnormalities)

A sensitivity analysis would have been performed excluding studies in which there was uncertainty if the specific inclusion criteria were met.

Studies specifically involving neutropenic patients or patients following chemotherapy were excluded.

### **5.2.2. *Types of interventions***

The use of G-CSF as an adjunct to antibiotics was assessed, including studies involving all doses of G-CSF administered intravenously or subcutaneously. Trials that allowed concurrent use of other therapies, including mechanical ventilation and immunomodulatory agents including steroids were included if they allowed equal access to such medications for patients in both arms of the trial.

### **5.2.3. *Types of outcome measures***

The primary outcome was 28 day mortality.

Secondary outcomes included:

1. In-hospital mortality;
2. Rate of mechanical ventilation;
3. Duration of intensive care unit (ICU) admission following randomization;
4. Duration of hospital admission following randomization; and
5. Adverse events, including the incidence of organ failure (ARDS, disseminated intravascular coagulation, acute renal failure, development of shock).

### **5.2.4. *Search strategy for identification of studies***

The following electronic databases was searched in mid-2003 and updated in 2004:

1. The Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library, Issue 1, 2004);
2. MEDLINE (January 1966 to March Week 1, 2004);
3. EMBASE (1988 to December 2003);
4. Online databases of clinical trials ([www.controlled-trials.com](http://www.controlled-trials.com));
5. Contact with corresponding authors;
6. Contact with the manufacturers and distributors of filgrastim (Amgen) and lenograstim (Chugai, Japan and Merck, Australia);
7. Reviews of citations in publications identified by the above strategies.

MEDLINE and CENTRAL using the following search strategy was combined with the highly sensitive search strategy suggested in the Cochrane Reviewers Handbook 4.1.5 [20] for identification of randomized clinical trials (

Table 5-1). Studies in all languages were included. Studies identified as randomized controlled trials in non-neutropenic adults were examined further for eligibility.

*Table 5-1: Search strategies for clinical trials of G-CSF in pneumonia*

MEDLINE (OVID) (January 1966 to March Week 1, 2004)

- #1 exp Community-Acquired Infections/
- #2 exp pneumonia/
- #3 exp respiratory tract infection/
- #4 exp granulocyte colony stimulating factor/
- #5 exp cross infection/
- #6 exp ventilators, mechanical/
- #7 community-acquired pneumonia.mp.
- #8 hospital acquired pneumonia.mp.
- #9 nosocomial pneumonia.mp.
- #10 ventilator associated pneumonia.mp.
- #11 lenograstim.mp.
- #12 filgrastim.mp.
- #13 pegfilgrastim.mp.
- #14 exp SEPSIS/
- #15 (#1 and (#2 or #3)) or #7
- #16 ((#5 or #6) and (#2 or #3)) or #8 or #9 or #10
- #17 #4 or #11 or #12 or #13
- #18 #17 and (#14 or #15 or #16)

EMBASE (WebSPIRS) (January 1990 to December 2003)

- #1 explode 'pneumonia-' / all subheadings
- #2 (pneumonia in ti) or (pneumonia in ab)
- #3 explode 'lower-respiratory-tract-infection' / all subheadings
- #4 #1 or #2 or #3
- #5 (granulocyte colony stimulating factor in ti) or (granulocyte colony stimulating factor in ab)
- #6 explode 'recombinant-granulocyte-colony-stimulating-factor' /
- #7 (lenograstim in ti) or (lenograstim in ab)
- #8 (filgrastim in ti) or (filgrastim in ab)
- #9 (pegfilgrastim in ti) or (pegfilgrastim in ab)
- #10 #5 or #6 or #7 or #8 or #9
- #11 #4 and #10
- #12 explode 'randomized-controlled-trial' / all subheadings
- #13 explode 'controlled-study' / all subheadings
- #14 explode 'single-blind-procedure' / all subheadings
- #15 explode 'double-blind-procedure' / all subheadings

#16 explode 'crossover-procedure' / all subheadings  
 #17 explode 'phase-3-clinical-trial' / all subheadings  
 #18 (randomi?ed controlled trial in ti) or (randomi?ed controlled trial in ab)  
 #19 ((random\* or placebo\* or double-blind\*)in ti) or ((random\* or placebo\* or double-blind\*)in ab)  
 #20 (controlled clinical trial\* in ti) or (controlled clinical trial\* in ab)  
 #21 #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20  
 #22 (nonhuman in der) not ((human in der)and (nonhuman in der))  
 #23 #21 not #22  
 #24 #11 and #23

### 5.3. Data extraction

Two reviewers independently extracted data. Disagreements were resolved by discussion.

Data that were extracted included, where possible:

- Description of participants and recruitment;
- Description of pathogens and antimicrobial resistance;
- Description of intervention and co-interventions;
- Description of control therapy;
- Description of antibiotics used;
- Methodological details, including criteria for quality assessment (below);
- Total number of participants in each arm of the trial;
- Study setting, in particular intensive care unit (ICU) versus non-ICU;
- Markers of duration of morbidity, including intubation, time to discharge from ICU and hospital;
- Mortality; in-hospital and 28 day;
- Adverse events, including organ dysfunction;
- Source of funding.

#### 5.3.1. *Assessment of study quality and analysis*

Both reviewers assessed study quality based on standard Cochrane criteria for the assessment of the following components of study design [21]:

- Generation of allocation sequence;
- Allocation concealment;



- Blinding;
- Loss to follow-up.

Studies of poor methodology were not excluded from the primary analysis.

Study population, intervention and outcome measures of each study were assessed to see if the pooling of results was feasible. Heterogeneity was tested using the chi square test. However, given the intrinsic heterogeneity of the study populations, random effects analysis was to be employed if pooling was still felt to be appropriate. It was intended that where appropriate, relative risk/odds ratios, event rates, time-to-event and risk difference and corresponding 95% confidence intervals would be calculated. Data was analyzed using Review Manager (version 4.2, Cochrane Collaboration).

It was intended that a sensitivity analysis would be performed excluding studies of low methodological quality, unpublished studies and studies where some ambiguity exists about whether they met inclusion criteria

Intended *a priori* subgroup analyses, where possible, were to be performed for:

- Age category (older than 65 years);
- Patients with specific chronic diseases (diabetes, renal failure, hazardous alcohol use, chronic obstructive airways disease) separately;
- Bacterial aetiology;
- Community-acquired pneumonia, hospital-acquired pneumonia and suspected and confirmed ventilator-associated pneumonia separately
- Patients with and without severe sepsis

Publication bias was to be assessed by the use of a "funnel plot".

## 5.4. Results

### 5.4.1. *Studies identified*

Following an MEDLINE (OVID) search, 93 studies were identified for screening. Of these, 87 studies were excluded (duplicate studies, animal studies, studies of

neonatal or paediatric patients, studies of neutropenic patients or patients following chemotherapy or transplantation, studies that did not assess G-CSF as therapy (mainly basic science articles), review articles, non-randomized controlled studies, studies that did not involve patients with pneumonia and studies of G-CSF prophylaxis rather than therapy). One additional study was identified using EMBASE (n = 81), online searches of clinical trials registers (n = eight) or CENTRAL (The Cochrane Library, Issue 1, 2004) (n = 11).

Thus, seven studies were identified for detailed evaluation. Correspondence with the study sponsors confirms that a then-unpublished study of G-CSF in pneumococcal pneumonia referred to in Nelson [22] refers to a subgroup of Root [23]. Of these six studies, one was not a randomized controlled trial [24]. One study [25] did not report clinical endpoints such as mortality and the rate of organ dysfunction and thus was excluded from further analysis.

The remaining five studies were all sponsored by Amgen, the manufacturers of filgrastim and are summarized in Table 5-2. All studies met the definitions of community-acquired pneumonia and confirmed hospital acquired pneumonia where indicated. Patients in the later two studies [23, 26] included patients that met our definition for severe sepsis.

An initial clinical trial [27] excluded patients with septic shock, regarded as high risk for developing ARDS, and was conducted in multiple sites in North America and Australia. It included only patients with community-acquired pneumonia requiring hospital admission. Although patients were required to have two risk factors that had been demonstrated to be associated with increased mortality, patients with septic shock were specifically excluded. In this large study (n = 756), filgrastim (300 mcg / day for 10 days) and control groups were balanced with regard to risk factors. The primary end point was a composite measure of clinical findings ("time to resolution of morbidity"), but other endpoints were reported.

A subsequent trial [22] included patients with community-acquired multilobar pneumonia, as the previous trial had suggested that this group, recognized as a group at higher risk for mortality, might have benefited from G-CSF. In this study (n =

480), there were fewer diabetics in the intervention group, but they were otherwise well balanced. The primary endpoint was a composite endpoint of organ dysfunction, empyema or death. Mortality and markers of adverse events, including organ dysfunction was reported.

A small trial [26] (n = 18) was conducted specifically in patients with pneumonia and septic shock primarily to evaluate safety. Patients were randomized 2:1 to filgrastim (300 mcg / day for five days) or placebo and included patients with both community-acquired and hospital-acquired pneumonia. Mortality at seven and 28 days as well as markers of adverse events including organ dysfunction was reported.

Following the safety analysis of the previous study, a large study [23](n = 701) evaluated filgrastim (300 mcg / day for five days) in patients with severe sepsis and community-acquired or hospital acquired pneumonia requiring admission to intensive care. Neutropenic patients and those who had received chemotherapy were not excluded. Intervention and control groups were well balanced at baseline. Mortality at 28 days and total adverse events were reported. Unpublished data regarding specific rates of organ dysfunction was provided by the study sponsors (Foote 2003).

A fifth trial was published in German as an abstract [28](n = 29); unpublished data was obtained from the authors (Kober T, personal communication). This study enrolled patients with hospital-acquired pneumonia but was terminated early due to poor enrolment. G-CSF was administered according to a weight-based protocol (300 mcg / day for patients < 75 kg, 480 mcg / day for patients > 75 kg; for up to seven days). Mortality at 15 days was the primary endpoint but 30-day mortality was also reported. Rates of serious adverse events were sought, as were rates of ARDS.

The bacterial aetiology was similar in all studies, with *Streptococcus pneumoniae* (*S. pneumoniae*) the most common organism found. In the studies of patients with septic shock, there was a greater proportion of *Staphylococcus aureus* compared to *Haemophilus influenzae* reflecting the more severe illness that accompanies infection with *S. aureus*. Gram negative organisms predominated in the study of patients with hospital-acquired pneumonia [28].

#### 5.4.2. *Methodological quality of included studies*

There was general agreement between the study reviewers; a number of minor issues regarding outcome measures were resolved by discussion.

The method of randomisation and generation of allocation sequence was only reported in one paper [23], but contact with the study authors and sponsors confirms that computer-generated randomisation lists and *a priori* numbered boxes were used in each centre in all studies (Foote M, personal communication). It is likely that blinding after allocation of healthcare providers and observers may have been incomplete as median white cell counts were much higher in the G-CSF treated groups [22, 27] but the degree of blinding was not formally assessed. Follow up was generally good but incomplete in between 3% to 7.9% of participants.

Table 5-2: Characteristics of included studies

Study	Methods	Participants	Interventions	Outcomes	Notes	Allocation concealment
Mansmann 2001	Single centre, double blinded RCT	Hospital-acquired pneumonia in ICU patients	G-CSF 300 mcg/d or 480 mcg/d for up to 7 days versus placebo	15 day mortality, safety, duration of antibiotic therapy		A
Nelson 1998	Multicentre, double blinded RCT	Community-acquired pneumonia in hospitalized adults.	G-CSF 300 mcg/d for 10 days versus placebo	Time to resolution of morbidity, 28 day mortality, time to ICU and hospital discharge, adverse events including organ dysfunction.		A
Nelson 2000	Multicentre, double blinded RCT	Community acquired multilobar pneumonia in hospitalized adults	G-CSF 300 mcg/d for 10 days versus placebo Placebo	28 day mortality, therapeutic failure, adverse events including organ dysfunction No difference in organ dysfunction		A
Root 2003	Multicentre, double blinded RCT	Confirmed hospital acquired or community acquired pneumonia with severe sepsis	G-CSF 300 mcg/day for 5 days versus placebo	28 day mortality, time to ICU discharge, adverse events		A
Wunderink 2001	Double blinded RCT in 3 centres in US	Confirmed hospital acquired or community acquired pneumonia with severe sepsis	G-CSF 300 mcg/d for 5 days versus placebo	Safety	Small study. heterogenous population.	A

## 5.5. Results

Five studies with a total of 1,984 patients were identified for further analysis.

### 5.5.1. *Mortality and other efficacy endpoints*

None of the studies demonstrated a statistically significant mortality benefit; a pooled estimate of efficacy similarly did not demonstrate a significant benefit associated with G-CSF. There was some heterogeneity amongst studies with regard to 28 day mortality ( $p = 0.14$ ) but this was not statistically significant. Pooling results across all five studies, the pooled odds ratio for 28 day mortality was 0.86 (random effects model), 95% confidence interval (CI) 0.56 to 1.31; Figure 5-1).

A number of the endpoints intended for analysis were not reported, including time to hospital discharge (no interquartile range reported in any study), the rate of intubation (only reported in Nelson, 1998 [27]), in-hospital mortality (only reported in Nelson 1998 [27] and [28]). Duration of ICU stay was not reported in one study [26]; in the remaining four studies, no differences were seen in median ICU stay which ranged from four days in both groups [27] to 16 days in both groups [28], reflecting the severity of illness of each study population

### 5.5.2. *Adverse events*

Overall, there was a reduction in adverse events that was not statistically significant (pooled odds ratio was 0.79; 95% CI 0.50 to 1.23; Figure 5-2). This was largely due to a reduction in rates of organ dysfunction, highest in the study of community-acquired pneumonia [27]. In that study, there was a significant decrease in rates of ARDS (OR 0.28; 95% CI 0.09 to 0.28) but this was not seen in subsequent studies; overall use of G-CSF was not associated with a reduction in ARDS (OR 0.92; 95% CI 0.44 to 1.93; Figure 5-3). When considering rates of individual organ dysfunction, there was a moderate decrease in the rates of disseminated intravascular coagulation (OR 0.74; 95% CI 0.38 to 1.44; Figure 5-4), acute renal failure (OR 0.79; 95% CI 0.48 to 1.31;

Figure 5-5) and incident septic shock (OR 0.61; 95% CI 0.34 to 1.09; Figure 5-6), but this was not statistically significant.

### **5.5.3. Community acquired pneumonia, hospital acquired pneumonia and ventilator-associated pneumonia**

Only two of the three studies that included patients with hospital-acquired pneumonia reported results by category. In the larger study [23], patients with hospital-acquired pneumonia constituted a minority (20%) of patients and were distributed evenly in intervention and placebo groups.

### **5.5.4. Presence of severe sepsis**

Outcome measures were not reported by the presence or absence of severe sepsis in the earlier trials [22, 27]. In these trials, however, septic shock was a specific exclusion; the pooled odds ratio of incident septic shock was 0.60 (95% CI 0.34 to 1.08). There was a moderate decrease in 28 day mortality in these trials (pooled OR 0.80; 95% CI 0.52 to 1.22) that was not statistically significant.

There was a trend to an increasing odds ratio of serious adverse events and organ dysfunction in the studies of patients with severe sepsis in filgrastim-treated patients [23, 26]. For total serious adverse events, the pooled odds ratio (random effects) in the studies of patients with severe sepsis was 1.10 (95% CI 0.82 to 1.49) compared to the other studies (0.72; 95% CI 0.51 to 1.01). Mortality was higher in the G-CSF groups in the trials of patients with severe sepsis (OR 1.13; 95% CI 0.82 to 1.57)

### **5.5.5. Other subgroup analyses**

Results were not reported by bacterial aetiology; it was not possible to draw conclusions on the efficacy of G-CSF in each of these groups. Subgroup analyses on patients with specific co-morbid conditions could not be performed due to a lack of data.

### **5.5.6. Other intended a priori analyses**

Given the small number of studies, a sensitivity analysis and a funnel plot were not performed. No individual study demonstrated a mortality benefit

## 5.6. Discussion

### 5.6.1. Efficacy

This analysis suggests that there is no clinically significant benefit associated with the use of G-CSF. Richard Root [23] suggested several reasons for the failure of previous animal work to demonstrate similar results in humans; invalid hypothesis, inadequate dosing or activity, improper study design or failed execution. He suggests that, primarily, delays in the administration of G-CSF to satisfy microbiological study criteria may have contributed to its negative result.

The original hypothesis suggested that G-CSF may benefit patients with acquired neutrophil dysfunction due to co-morbid conditions such as diabetes, alcohol and renal failure, or with infections due to intracellular organisms such as *B. pseudomallei*. However, this specific question could not be addressed from the data available.

### 5.6.2. Adverse events

These results suggest that G-CSF does not increase the rates of immunologically mediated organ dysfunction by a clinically significant extent. A theoretical concern has been that the use of G-CSF may increase the rate of immunologically mediated end-organ dysfunction, such as acute respiratory distress syndrome (ARDS), to which patients with severe sepsis are particularly prone.

There was a trend towards an increasing risk of organ dysfunction in patients more severely unwell and treated with G-CSF, suggesting that this may have been due to an exacerbation of immunologically mediated disease in patients more at risk of developing organ dysfunction. However, this was not statistically significant in any study and remains speculative. The trend towards a reduction in incident septic shock in patients without this complication at study enrolment may suggest that G-CSF may be operating to prevent this complication.



### **5.6.3. *Decision to pool data from trials***

Despite the clinical heterogeneity present between the study populations, it was felt appropriate to pool the results to obtain an overall estimate of efficacy. Although the inclusion criteria were different, these patients all had varying severity of the same disease and thus the relative efficacy might be expected to remain the same in each group, although the absolute benefit (or harm) might be expected to vary with the mortality of the population studied. Similarly, it was felt that, although patients with severe sepsis may be a higher risk of developing this complication, the mechanism of end-organ dysfunction was the same.

### **5.6.4. *Possible sources of bias***

Given the lack of effect demonstrated by the individual published trials, a significant publication bias is unlikely to be operating. However, it is possible that trials may have been withheld from publication due to harmful effects. There is no specific information suggesting this possibility.

## **5.7. Conclusions**

### **5.7.1. *Implications for practice***

There is currently no evidence supporting the routine use of G-CSF in the treatment of pneumonia. There is no data on the use of G-CSF in subgroups such as diabetics that may manifest functional neutrophil deficits. No methodological issues or biases that may have influenced our pooled results were identified

### **5.7.2. *Implications for research***

Clinical trials where G-CSF was administered earlier in the course of disease or perhaps even prophylactically to prevent hospital acquired infection in high risk patients might be of interest. Similarly, studies of G-CSF for organisms where neutrophil function may be more important, or in hosts with co-morbid illnesses that may manifest acquired neutrophil dysfunction may be of interest.

Researchers are encouraged to report methodological parameters, such as generation of allocation sequence, allocation concealment, blinding and loss to follow-up as

well as standardized outcome measures. A useful parameter that may have an impact on outcome, the proportion of patients on appropriate antibiotics within the first 24 hours of therapy, should also be reported.

Figure 5-1: G-CSF in pneumonia meta-analysis: 28 day mortality forest plot

Review: Granulocyte-Colony Stimulating Factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults  
 Comparison: 01 Mortality  
 Outcome: 01 28 day mortality

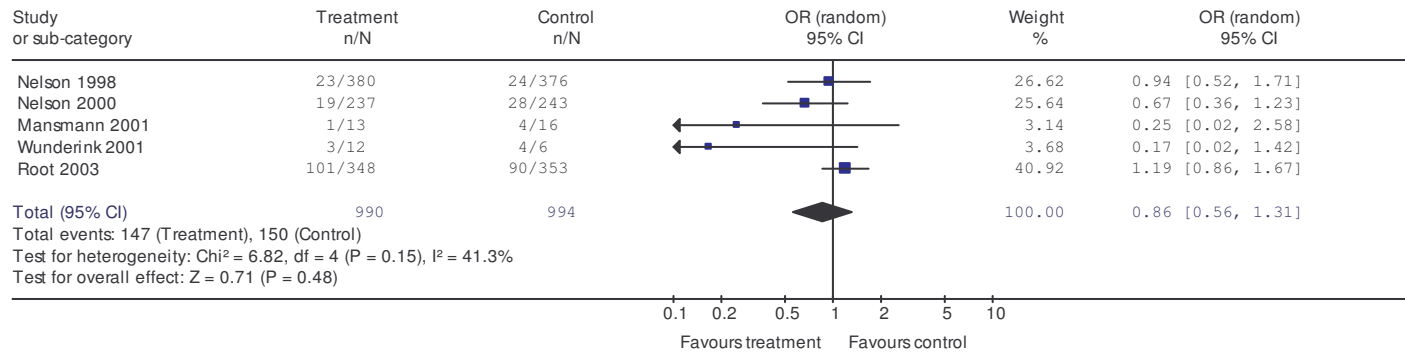


Figure 5-2: G-CSF in pneumonia meta-analysis: all adverse events forest plot

Review: Granulocyte-Colony Stimulating Factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults  
 Comparison: 02 Adverse events  
 Outcome: 01 Any adverse event (including organ dysfunction)

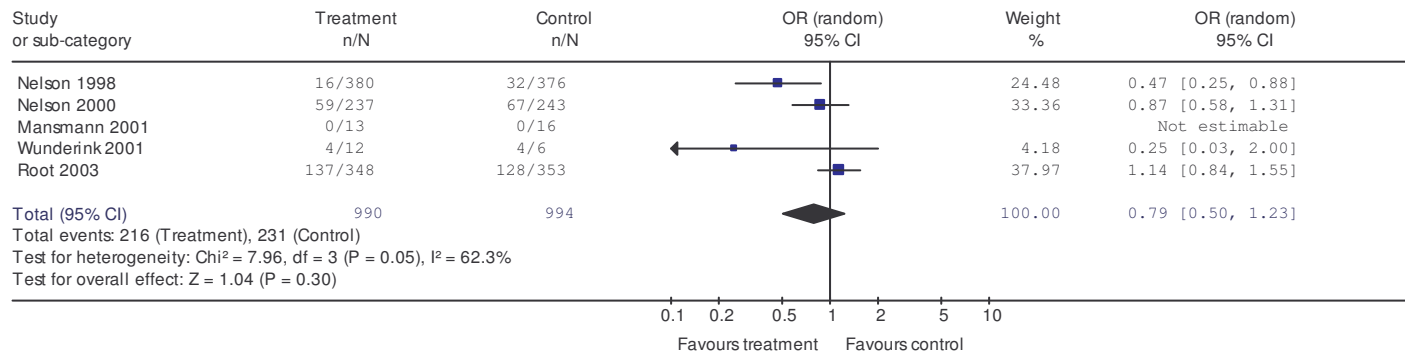


Figure 5-3: G-CSF in pneumonia meta-analysis: Incident acute respiratory distress syndrome forest plot

Review: Granulocyte-Colony Stimulating Factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults  
 Comparison: 02 Adverse events  
 Outcome: 02 Adult respiratory distress syndrome

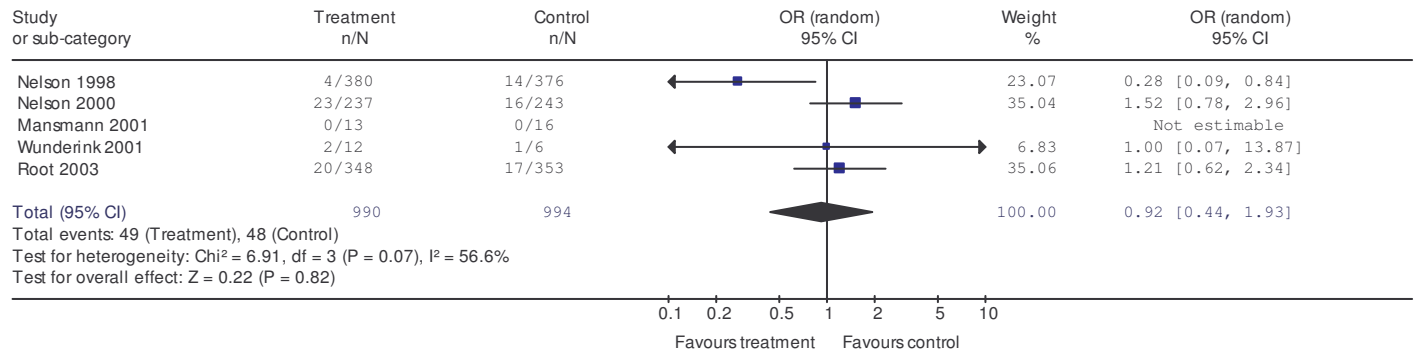


Figure 5-4: G-CSF in pneumonia meta-analysis: disseminated intravascular coagulation forest plot

Review: Granulocyte-Colony Stimulating Factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults  
 Comparison: 02 Adverse events  
 Outcome: 03 Disseminated intravascular coagulation

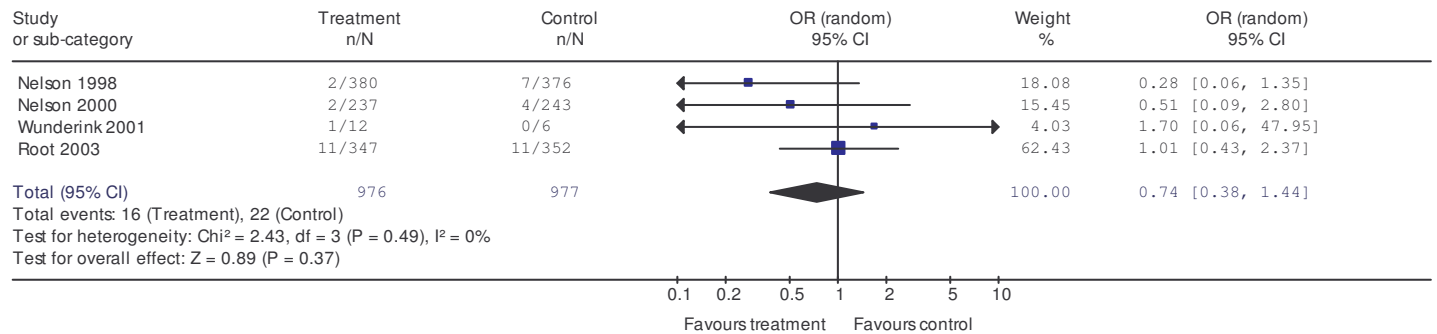


Figure 5-5: G-CSF in pneumonia meta-analysis: acute renal failure forest plot

Review: Granulocyte-Colony Stimulating Factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults  
 Comparison: 02 Adverse events  
 Outcome: 04 Acute renal failure

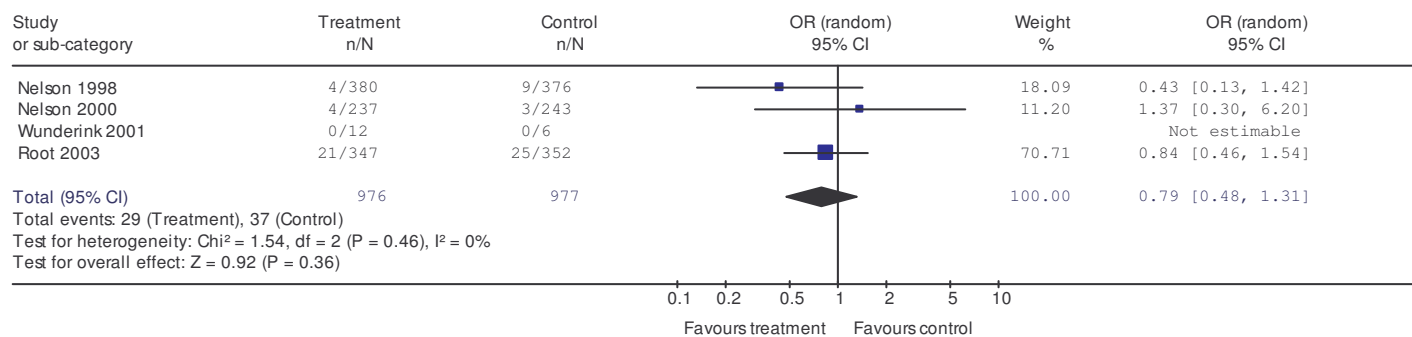
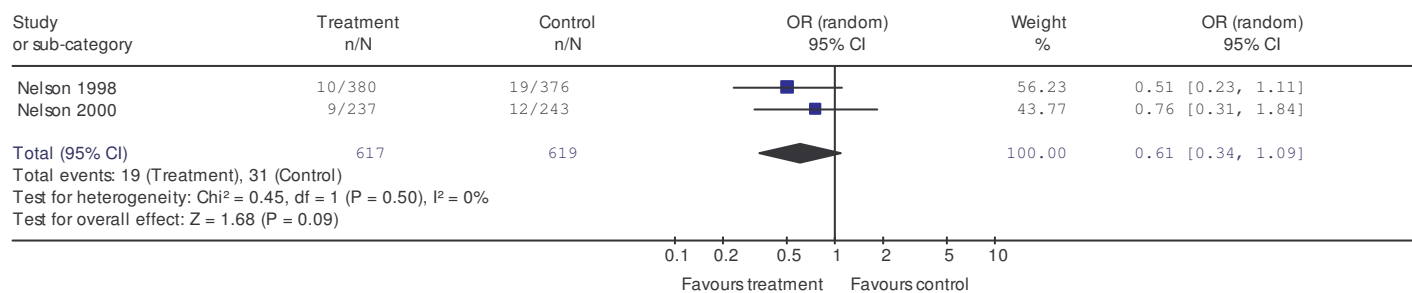


Figure 5-6: G-CSF in pneumonia meta-analysis: incident septic shock forest plot

Review: Granulocyte-Colony Stimulating Factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults  
 Comparison: 02 Adverse events  
 Outcome: 05 Septic shock



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## **6. An experiment that cannot be done - G-CSF in the treatment of severe melioidosis in Australia**

### **6.1. Introduction**

As outlined previously, a clinical decision was made at the Royal Darwin Hospital to introduce granulocyte-colony stimulating factor (G-CSF) in the management of patients with melioidosis and septic shock based on evidence available in late 1998. This included theoretical and animal data, a clinical trial published in 1998, the relative safety of G-CSF and the nearly universal mortality associated with septic shock due to melioidosis.

There was a dramatic reduction in mortality from severe melioidosis (from 95% to 10%,  $p < 0.001$ ) and a more modest reduction in mortality from septic shock due to other pathogens [1]. However, a number of confounding factors were present and may partially explain the fall in mortality. The introduction of G-CSF was coincident with an appointment of specialist in intensive care medicine, where previously the unit had been supervised by anaesthetists and physicians. This resulted in significant changes to management protocols, including the more aggressive use of haemodynamic monitoring, empiric antibiotic protocols, the adoption of a closed intensive care model and early enteric feeding.

The mortality benefits were in contrast to other published data outlined in the previous chapter. A clinical trial of G-CSF in severe melioidosis was therefore proposed. The trial was to have taken the form of a randomized controlled trial (RCT) in which eligible patients were randomized to either G-CSF or placebo. The end-points to be evaluated would have been death from any cause or deterioration to catecholamine-resistant shock. Those who declined to participate in the study would be treated with the usual protocol, which involved the routine use of G-CSF.

### **6.2. Methods:**

In collaboration with infectious diseases physicians and the intensivist, a study protocol for a clinical trial of G-CSF in melioidosis was drafted. It was evident

during the planning for this trial that complex ethical issues were involved and a dialogue was established with a clinical ethicist and interim member of the ethics committee.

This study protocol was discussed extensively with junior and senior medical staff and medical researchers in Darwin as well as in correspondence with other colleagues in northern Australia and internationally. A review of the literature was performed to better define the ethical issues involved and explore methods that such problems had been approached by others.

### **6.3. Results**

#### **6.3.1. Clinical, personal and patient equipoise**

Discussions of the ethics of RCTs emphasise the need for a state of equipoise to exist before a trial can proceed. Equipoise is variously defined - common interpretations are that it consists of uncertainty that rests with researchers or the expert community as a whole [2, 3]. In this case there was disagreement within the medical community about the value of G-CSF. This could be seen as evidence that a trial of G-CSF in severe melioidosis was both necessary and ethically justifiable.

Despite the apparent presence of equipoise, many local experts continued to be uneasy about the trial, as they felt that the risks to patients of foregoing G-CSF were too great. Indeed, RCTs are mostly advocated in situations where there is only a moderate or small effect size rather than the large effect size that was possible in this case. Peto suggests that in these circumstances randomized trials may be unnecessary [4]. For example, there has never been a randomized, controlled trial of the use of penicillin for syphilis, but trials involving its withdrawal are rightly regarded as unethical. Other analogous situations where previous evidence may have been sufficient to disturb the state of equipoise have included neonatal extracorporeal membrane oxygenation (ECMO) [5], a novel therapy for in gastrointestinal stromal tumours [6] and quinacrine for Creutzfeldt-Jacob disease [7];

In a 1991 paper, Royall attempted to deal with this issue by distinguishing truly “experimental” trials from unethical “demonstration” trials, with the latter designed to display the superiority of already-successful treatments in a more convincing

fashion [8]. Our situation demonstrates the difficulties with this concept - does our potentially confounded series constitute sufficient evidence of effectiveness?

Traditionally, equipoise has been viewed from the rather paternalistic viewpoint of the treating clinicians and the medical community. However, there is a growing body of work discussing equipoise from the perspective of patients or the wider community [9-11]. For example, Brody has argued that patient equipoise might be present if “a reasonable person of an average degree of altruism and risk adversiveness might consent to being randomized.” This is analogous to clinical equipoise being determined by the normative judgment of a community of physicians [12].

In our case, information about the apparent value of G-CSF was already well-known within the community, with G-CSF being described locally as the “Wonder Drug [that] Beats Killer Bug” (Sunday Territorian, 23 Sept 2001; Figure 6-1). It is difficult to see how the survival statistics (95% vs 10% mortality) would lead anybody other than the most extreme altruist to agree to participate in the proposed trial, no matter what the experts thought.

Figure 6-1: Who wouldn't want this?



### 6.3.2. How can this question be answered?

The proposal for a local trial was eventually abandoned because of these ethical considerations. Yet the question remains – is G-CSF of any value in the treatment of septic shock associated with melioidosis? A number of variations to the proposed trial design were suggested in the hope that they would decrease the risks to participants and/or the number of people who might be harmed by foregoing G-CSF.

One method would be by trialing the current dosing regime against shorter, delayed, or less potent dosing regimes rather than placebo. This is similar to the approaches used in the developing-world trials of AZT in HIV prevention [13]. Because smaller doses of the agents are likely to have some effect, a larger trial would be required, but the risks to the individual patient would be decreased. This approach did not prove practical because of the need for a greater sample size than was feasible.

A second method would involve the use of surrogate end-points that might allow deteriorating patients to be withdrawn from the trial early, an approach used in a trial of steroids in pneumocystis pneumonia [14]. Unfortunately, appropriate surrogate end-points have not been well defined in trials involving septic shock.

A third method of making this study safer would be to use an adaptive trial design such as “play the winner” [15] where allocation of patients to each arm of the trial is based on previous results of the intervention. These designs have been used in trials of extracorporeal membrane oxygenation [16]. Although they limit the number of possible adverse outcomes, they are unable to prevent them altogether. Importantly, they do not limit the risks to an individual patient who enters early in the trial.

A fourth method would be through the exclusion of the most seriously unwell patients. This would potentially decrease the risk of omitting G-CSF but would also increase the number of patients required for the study, and decrease the applicability of the study to seriously ill patients.

It is possible that future advances in the understanding of melioidosis will show other plausible reasons for the marked fall in mortality of these patients at RDH. If this were the case, G-CSF might again come to be seen as an optional part of their treatment, and a greater degree of equipoise might allow G-CSF to be more formally evaluated within RDH. However at present it does not appear possible to limit the risks to individual patients within the current constraints of sample size.

Locally and elsewhere in Australia, researchers have attempted to use animal and *in vitro* models to explore the role of G-CSF in acute melioidosis [17]. However, given that such models [18] have failed to translate into improved outcomes in human

trials of G-CSF in non-neutropenic infection, such evidence is no substitute for a clinical trial.

More than half of the Australian cases of melioidosis are seen at our institution. Thus, if such a trial cannot be performed at RDH, it is not likely to be feasible elsewhere within Australia. In conjunction with colleagues in Thailand, such a trial was proposed. Although a discussion of the ethics of clinical trials in developing countries is beyond the scope of this paper, recent guidelines suggest that trials of new forms of therapy may be ethical in developing countries if the results might benefit both the participants of the trial and others in the community [19]. G-CSF is a relatively expensive agent but its use would be sustainable in this setting if a substantial benefit could be shown. However, the results of such a trial, in a developing world context, may not be directly applicable to our patient population in Australia.

#### **6.4. Conclusion**

A randomized controlled trial of G-CSF in severe melioidosis in Darwin was not feasible because it was felt that our observational evidence would have convinced many in the community of its effectiveness. In addition, an alternative trial design could not be identified that would ensure the safety of potential trial participants. Most trials take place in the context of limited but promising evidence of a modest benefit. This situation illustrates the problems in defining the state of uncertainty necessary for the conduct of an ethical trial when a substantial benefit has been observed, however flawed this evidence might be.

This use of G-CSF remains unproven and its adoption in the treatment of septic shock associated with melioidosis outside our institution seems unlikely in the absence of additional evidence. A clinical trial of G-CSF has been proposed in Thailand; the results of this trial may prompt a re-evaluation of its use in Australia.

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## **7. Granulocyte-colony stimulating factor and an *in vitro* whole blood model of melioidosis**

### **7.1. Introduction**

In previous chapters, it was theorized that patients with risk factors for acquired neutrophil dysfunction, infected with an intracellular bacterium such as *B. pseudomallei*, may have benefited from the use of G-CSF. In this study, a whole blood *in vitro* model was formulated to explore possible mechanisms of G-CSF action.

### **7.2. Materials and Methods**

#### **7.2.1. Isolate:**

A clinical isolate of *B. pseudomallei*, identified by colonial morphology, biochemical reactions and agglutination with specific antiserum was isolated from the sputum of an adult with pneumonia. This isolate had been stored in Todd Hewitt broth with 30% glycerol at  $-70^{\circ}\text{C}$ . Subcultures were grown to log phase in Luria Bertani broth at  $37^{\circ}\text{C}$ . Bacteria were diluted with phosphate buffered saline (PBS) before being adjusted to a concentration of  $2.0 \times 10^8$  cfu/mL by spectrophotometric standardization at 600nm.

#### **7.2.2. Pilot studies:**

Previous studies were performed that were consistent with those by other researchers; they had shown that neutrophils efficiently phagocytosed fluorescein-labelled *B. pseudomallei* [1] and this high phagocytosis rate did not improve following co-incubation with G-CSF. Most of the bactericidal ability of whole blood occurred early (within 60-90 minutes), with regrowth of bacteria after this time [2]. We also demonstrated that G-CSF did not have a direct inhibitory effect on *B. pseudomallei* growth and neutrophils remained viable using the trypan blue exclusion technique. A mid-log growth point was established by serial measurements of optical density.



### 7.2.3. *Study subjects:*

Healthy volunteers (controls) were recruited from staff at the Menzies School of Health Research. Patients on haemodialysis were recruited from a local community dialysis centre. Patients with type 2 diabetes mellitus were recruited from hospital inpatients and outpatients. Whole blood were taken by venepuncture, heparinized and immediately processed following transport to the Menzies School of Health Research. All subjects were *B. pseudomallei* antibody negative by the indirect haemagglutination assay.

### 7.2.4. *G-CSF:*

Filgrastim (Amgen, Thousand Oaks, CA) was diluted with PBS to a concentration of 300ng/mL. Whole blood and sera were co-incubated with 5 ng of G-CSF for 20 minutes at 37°C prior to inoculation with *B. pseudomallei*.

### 7.2.5. *Procedure:*

Whole blood (500µL) and same subject serum controls (250µL with 250µL PBS), with and without G-CSF, were then inoculated with a mid-log culture of *B. pseudomallei* (10µL), calculated to give a multiplicity of infection (bacteria:cell) ratio of 1:1. Inoculated samples were incubated at 37°C for 60-90 minutes in a shaking orbital. Assays using pooled human serum (*B. pseudomallei* antibody-negative) as a control were conducted in parallel. An aliquot (25µL) was added to sterile water (225µL) to lyse all cellular contents. Viable counts were determined at baseline and at 60-90 minutes by using standard colony counting techniques using serial dilutions in PBS and overnight incubation on HBA. Bactericidal activity was calculated comparing viable colony forming units in blood at 60-90 minutes compared to baseline.

### 7.2.6. *Ethics and statistics:*

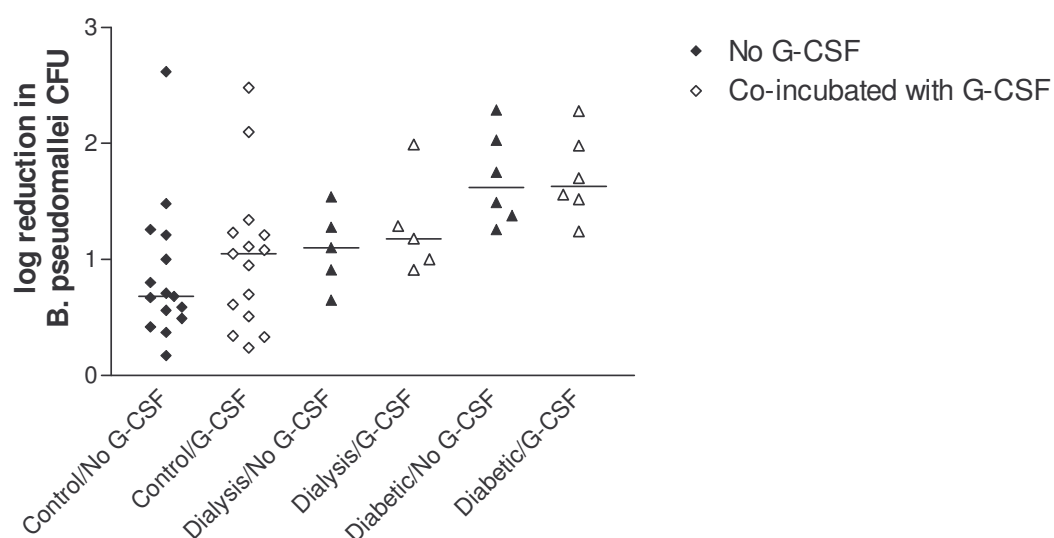
The study was approved by the Human Research Ethics Committee of the Menzies School of Health Research and Territory Health Services and conformed to the guidelines of the Declaration of Helsinki. Informed consent was obtained from all participants. Statistical analysis was performed using Prism 3.02 (GraphPad Software); paired and unpaired Student's t-tests on geometric means (GM) were performed where appropriate.

### 7.3. Results

Blood and serum from 15 healthy controls (HC), 5 dialysis patients and 6 diabetic patients were tested. Whole blood from diabetic patients had a higher reduction in bacterial counts (GM log 1.70, 95% CI: 0.53, 1.20) compared with the healthy controls (log 0.87, 95% CI: 0.67, 1.37) and dialysis patients (log 1.10, 95% CI 1.28, 2.12; ANOVA  $p=0.01$ ). In contrast, there was an increase in bacterial counts when bacteria were incubated with serum alone (control GM log 0.21, dialysis log 0.13, diabetic 0.05) but this was not statistically significant (ANOVA;  $p=0.14$ )

There was no significant change in whole blood bactericidal ability following co-incubation with G-CSF in healthy controls (difference in geometric means log -0.15; paired t-test  $p=0.28$ ), dialysis patients (-0.17,  $p=0.12$ ) or diabetic patients (-0.01,  $p=0.84$ ). Results for the whole blood bactericidal activity of all groups are graphically illustrated in figure 1.

Figure 7-1: Whole blood bactericidal activity, measured by reduction in bacterial counts, of control, dialysis and diabetic subjects without/with co-incubation with G-CSF



#### 7.4. Discussion

Whole blood assays have provided models for *Vibrio vulnificus* in patients with chronic liver disease [3] as well as intracellular *Mycobacterium tuberculosis* and *Neisseria meningitidis*. However, using this model we have been unable to demonstrate significant differences with co-incubation with G-CSF, or between patients with risk factors for melioidosis and healthy controls.

Our rationale for using G-CSF in patients with septic shock due to melioidosis was based on previous work that had suggested that *B. pseudomallei* was able to survive and multiply within cells, including neutrophils [2]; that diabetes and chronic renal failure, associated with melioidosis [4] are also associated with functional neutrophil defects; G-CSF had been shown to improve outcomes from sepsis in animal models and improve neutrophil function *in vitro* [5]. In addition, clinical evidence available at that time suggested that G-CSF may be of benefit in subgroups of patient with pneumonia [6], but this was not confirmed by subsequent trials [7, 8].

Previous studies of bactericidal activity of phagocytes for *B. pseudomallei* have shown conflicting results. Although Egan *et al* demonstrated increased phagocytosis with opsonization with complement, bacterial killing was poor at 60 minutes [1], similar to the poor early killing noted in another study [9].

However, these study results, demonstrating moderate bactericidal activity are more similar to that of Razak *et al*, who demonstrated killing rates of 87-92% after 30 minutes which was dependent on opsonization by heat-labile serum proteins (probably alternative complement pathway proteins) [10]. In addition, Jones [2] and Pruksachartvuthi [9] also demonstrated the growth of *B. pseudomallei* after extended incubation; the significance of these results is uncertain and we did not consider this in our study.

We felt that use of an *in vitro* whole blood model may have been more physiological, as bactericidal ability may be influenced by the humoral components of blood. In our study examining the early killing of *B. pseudomallei*, we found that there was approximately a 10-fold reduction in bacterial counts at 60-90 minutes

with variation within the assay greater than any potential difference between the groups. Although our small numbers had limited power, the lack of a trend, together with the substantial intra-group variation, suggests that a true difference was unlikely within the limitations of this study design.

There are a number of possible reasons for this. In contrast to *Vibrio vulnificus* [3], *B. pseudomallei* has a number of properties that may make this model less suitable. Resistance of *Vibrio vulnificus* is probably dependent on its ability to evade phagocytosis, compared with *B. pseudomallei* which evades phagocyte killing intracellularly. In addition, human serum has poor activity against *B. pseudomallei*, which tends to grow in the extracellular compartment during the assay.

We are unable to explain why diabetic patients, in contrast to our hypothesis demonstrated greater bactericidal activity than healthy controls. The growth of *B. pseudomallei* in serum was less in diabetic patients, but the magnitude of this difference is insufficient to account for the difference in whole blood bactericidal activity. A study seemed to demonstrate a direct inhibitory effect of insulin on the growth of *B. pseudomallei*, but this was later found to be due to a preservative [11].

In non-neutropenic infection, G-CSF may act through alternative mechanisms. *In vitro* and animal models have demonstrated synergy between G-CSF and antibiotics [12] which may be due to an increase in the intracellular concentrations of antibiotics. Another postulated effect of G-CSF is in attenuating the inflammatory cytokine profile. Animal and human studies have shown a decrease in circulating proinflammatory cytokines such as IL-2 and IFN- $\gamma$  and an increase in the anti-inflammatory cytokines IL-1ra, sTNF-R and IL-10 [13].

Our study has several important limitations. G-CSF may have priming effects on neutrophils. Previous studies have demonstrated *in vitro* pre-incubation with PMN (as used in this study) as well as *in vivo* pre-dosing of human volunteers increased neutrophil function [5]. A group has recently published results of adjuvant pre-dosing of G-CSF in an established mouse model [14, 15], but whether such effects may be different in humans with melioidosis requires further study. We only included patients that did not have intercurrent illness; the cytokine milieu is vastly

different during severe sepsis than at baseline, and the acquired neutrophil dysfunction associated with this process may be reversed by the use of G-CSF.

In this study, we have been unable to demonstrate that there are baseline differences between patients with risk factors for melioidosis and controls, or that G-CSF augments the bactericidal ability of blood at a short incubation in an *in vitro* model. Based on our clinical evidence, it is possible that the functional defects in neutrophils present in sepsis and accentuated by diabetes, alcohol or renal failure may still be important in the pathogenesis of melioidosis, but therapeutic G-CSF also may be operating by other mechanisms.

### **7.5. Other laboratory work**

In addition to *in vitro* work, work was performed in a G-CSF gene knockout mouse model [16]. If susceptibility to *B. pseudomallei* could be demonstrated in comparison to wild-type mice and resistance restored by the use of therapeutic G-CSF, this could provide evidence of the key role of G-CSF in resistance to melioidosis.

The liver/spleen bacterial loads in G-CSF gene knockout mice (G-CSF *-/-*) were examined at 36 hours after intraperitoneal inoculation of approximately 400 colony forming units of *B. pseudomallei*. Ethical approval for these studies was obtained from the Animal Research Ethics Committee of the University of Melbourne. Mice were monitored daily for signs of distress and the experiment terminated if necessary to ensure a humane endpoint.

In initial experiments, G-CSF *-/-* mice (n=10) had higher liver/spleen bacterial loads than C57B/6 controls (n=10; geometric mean titre  $10^{7.50}$  vs  $10^{4.23}$ ). The effect of pre-treatment and treatment with G-CSF (50-250 $\mu$ g/kg, up to four days prior to inoculation with *B. pseudomallei*) was then assessed. Treatment or pretreatment with G-CSF did not appear to alter liver/spleen bacterial loads. This was not accounted for by variation in the bacterial inoculum. However, it was later discovered that G-CSF administered to these mice may have been inactivated in the process of dilution, casting doubt over the validity of these results.

In view of recently published work demonstrating no clinical benefit associated with G-CSF over antibiotics alone in a Balb/c mouse model of melioidosis [14], these experiments were abandoned.

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## **8. C-Reactive protein in the diagnosis of melioidosis**

### **8.1. Introduction**

Serum C-reactive protein (CRP) is a simple, rapid marker of the acute phase reaction that is elevated in inflammatory reactions and tissue damage. It is a commonly used clinical marker of bacterial sepsis as well as non-infectious inflammatory states such as rheumatoid arthritis [1-3]. A previous study demonstrated that CRP levels were a sensitive marker for clinical melioidosis, with all patients demonstrating significant elevations in levels [4].

In this study, the utility of serum CRP levels in the diagnosis of melioidosis was reviewed. Its role as a prognostic marker for mortality was explored, particularly as a risk stratifying tool to identify patients that may benefit from immunomodulatory therapies such as G-CSF

### **8.2. Methods**

Patients with culture-confirmed melioidosis in the endemic “Top End” of the Northern Territory of Australia have been studied prospectively since October, 1989. Treatment followed established protocols [5]; an intensive phase of trimethoprim/ sulfamethoxazole (TMP-SMX) with intravenous antibiotics (mostly ceftazidime but more recently meropenem in critically ill patients) for at least 14 days, followed by an eradication phase of oral antibiotics (usually TMP-SMX) for at least 3 months.

Chronic disease was defined as illness with symptoms for longer than 2 months duration on presentation. Relapsed disease was defined as a new presentation with acute culture-confirmed melioidosis after the resolution of symptoms and completion of therapy for the previous episode. Severe sepsis was defined by conventional definitions [6]. The admission period was defined as within 48 hours of admission. Positive serology was defined as an indirect haemagglutination titer of 1:80 greater. Mortality was defined as death attributable to melioidosis occurring during the hospital admission.

Assays for serum C-reactive protein (Ortho-clinical Diagnostics, Johnson and Johnson, Rochester NY) were performed at the Royal Darwin Hospital with a normal range of less than 7g/mL (0.7mg/dL). “Mildly elevated” levels were defined as a level of between 7 and 50mg/L as this range may have resulted in clinical uncertainty as to its significance.

Statistical tests were performed using Intercooled Stata 7.0 (College Station, Texas, United States). As the distribution of CRP levels was skewed, a Mann Whitney U non-parametric test was performed to compare groups for univariate analysis. Candidate variables identified at the 0.05 significance level were considered together for multiple variable regression analysis where CRP levels were considered in quartiles. Ethical clearance for this review was obtained from the Human Research Ethics Committee of the Menzies School of Health Research and the Northern Territory Department of Human Services.

### **8.3. Results**

Of 344 patients with culture-confirmed melioidosis presenting between December 1989 and September, 2002, 218 patients had CRP levels determined during hospital admission and 175 patients had CRP levels performed within 48 hours of admission. Of the 175 patients, 92.6% had acute melioidosis and 7.4% had chronic melioidosis. The proportions of patients with pneumonia (55.4%), and abscesses of prostate, liver or spleen (20.6%) were representative of the wider group [5], including those that did not have CRP levels measured on admission or during admission.

The median CRP during the admission period was 164 mg/L with an interquartile range (IQR) of 59 to 286 mg/L. Of these patients, 12% (n=21) did not have elevated CRP levels above the normal range, including two patients with severe sepsis. Of these 21 patients, the majority (n=15) were performed on the day of admission. A further 20 patients (11.4%) only had mildly elevated CRP levels on admission, including 10 patients with CRP levels performed on the day of admission. Six patients (14%) with severe sepsis had normal or mildly elevated CRP levels. Fifteen

patients presented with relapsed disease, of which two patients had CRP levels within the normal range and another two in the mildly elevated range.

Univariate analysis suggested patients with diabetes, chronic lung disease, bacteraemia, severe sepsis and positive serology had higher median CRP levels (Table 8-1). There were no significant differences when considering median CRP levels by age, gender, ethnicity, history of excess alcohol intake or presence of chronic renal disease.

Table 8-1: Clinical features and C-reactive protein levels during admission period

Morbidity		Number of patients	Median CRP (IQR); mg/L	Significance
Diabetes	Present	75	194 (85, 305)	0.007
	Absent	100	136 (14, 252)	
Chronic Lung Disease	Present	55	206 (128, 296)	0.003
	Absent	120	103 (36, 264)	
Severe sepsis	Present	43	299 (107, 373)	<0.001 <sup>a</sup>
	Absent	132	117 (36, 234)	
Blood culture	Positive	99	239 (107, 320)	<0.001 <sup>a</sup>
	Negative	74	71 (10, 179)	
Presentation	Acute	154	169 (69, 291)	<0.001
	Chronic	13	29 (1, 74)	
Serology	Positive	77	197 (88,296)	0.023
	Negative	83	120 (24, 258)	

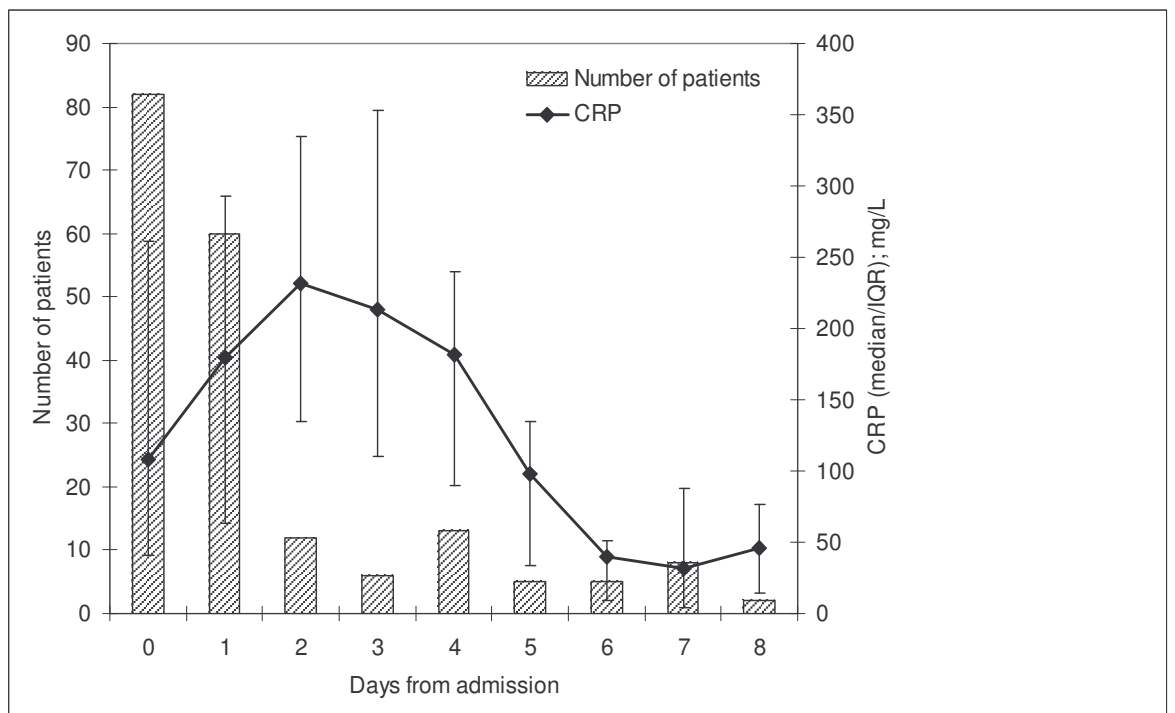
<sup>a</sup> Associated with higher quartile of CRP in a multiple variable model

Patients with chronic melioidosis had a lower median CRP; as this represented a different clinical manifestation, only patients with acute melioidosis were considered in the multiple variable analysis. Multiple variable regression suggested that only severe sepsis, bacteraemia and the presence of pre-existing chronic obstructive airways disease was associated with a higher quartile of CRP.

Of the 20 patients that died in this study, one (5%) had a normal CRP level and three (15%) had mildly elevated CRP levels. Patients who died had higher median CRP levels, but in a multiple variable analysis adjusting for the presence of severe sepsis and positive blood cultures, this was no longer significant with the presence of severe sepsis most strongly associated with mortality. In patients with the highest quartile of CRP levels, the mortality was 25.6%, higher than in the other quartiles (7.0%).

When considering serial CRP levels in the 218 patients who had CRP levels performed during hospital admission, the median CRP tended to increase in first few days before falling to lower levels after 6-8 days (Figure 8-1). Smaller numbers within subgroups precluded meaningful analysis, but a similar pattern was observed when comparing patients with pneumonia and patients with internal collections such as prostatic, liver or spleen abscesses (data not shown).

Figure 8-1: Distribution of C-reactive protein levels (median and interquartile range (IQR)) by time from admission



#### 8.4. Discussion

C-reactive protein, an acute phase protein, is synthesized by hepatocytes mainly in response to interleukin 6 (IL-6) [1] and acts as an opsonin, binding to polysaccharides and other molecules present in many pathogens. The secretion of CRP begins within 4-6 hrs of an inflammatory stimulus and may peak at levels up to 1000 times above the normal range after 36 to 50 hours [2]. Levels of CRP are a commonly used clinical tool in the diagnosis and monitoring of bacterial infection with an emerging role being defined in ruling out severe infection in different settings [2, 7].

In the only previous clinical study of CRP in melioidosis, serum CRP was reported to correlate with disease severity [4]. In all 46 patients with clinical melioidosis, which included 33 culture-confirmed cases, serum levels of CRP were elevated above 50mg/L at the time of diagnosis. In the majority of patients with an uncomplicated course, CRP levels decreased after two days of treatment and reached normal levels after a mean time of 29 days (range 12-52 days). Persistent elevation in four patients was attributed to undiagnosed sites of infection or inadequate treatment. Relapse was described in three patients and was associated with elevated CRP levels in the absence of fever or an elevated white cell count. A similar study by Smith et al in Thailand found that levels of another inflammatory marker, procalcitonin, were not sensitive for mild disease but invariably elevated in severe melioidosis, and were higher still in patients who ultimately died [8].

This larger study, involving only patients with culture-confirmed melioidosis, demonstrates that although CRP levels generally reflect the severity of infection, it is not a sensitive measure for this serious infection and cannot be relied upon to exclude the presence of this disease, mirroring studies examining the role of CRP in serious infections in children and adults [9-11]. Even amongst patients with severely septic presentations and patients that ultimately died, a significant proportion had CRP levels in the normal or mildly elevated range. Similarly, as a tool for detecting relapsed disease, a significant proportion did not have significant elevations of CRP within 48 hours of their second presentation.

The longitudinal analysis of CRP levels following admission should be interpreted with caution as there may be a significant testing bias operating, particularly following the immediate admission period. It was found that CRP levels fell to mildly elevated levels over the first week of therapy. Another limitation of this study is that the duration of illness prior to presentation was unable to be controlled for, apart from acute and chronic presentations.

The finding of an association between underlying chronic obstructive airways disease and higher CRP levels may reflect an increased severity of illness, particularly pneumonia, in patients with underlying lung disease. In addition, work done in Australian Aboriginal populations suggests that the chronic low-grade infection associated with chronic obstructive airways disease may lead to moderate elevations in CRP levels (Maguire G, unpublished data).

Interleukin 6, the stimulus for CRP production, is elevated in infection with *B pseudomallei* along with pro-inflammatory cytokines such as TNF and IL-8 [12]. A study of 172 Thai adults with melioidosis showed that IL-6, IL-10 and APACHE 2 scores were all independently associated with mortality [13]. Although the highest quartile of CRP was associated with a higher mortality, this was not an independent predictor when adjusted for the presence of severe sepsis.

In this study, it was found that normal or mildly elevated CRP levels cannot exclude melioidosis, either on first admission or at relapse. CRP levels tend to fall during the first week of admission. Although inflammatory cytokines have been shown to be independent predictors of mortality, CRP levels largely reflect the septic process and appeared to be inferior to clinical definitions of sepsis severity as a risk-stratifying tool.

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## **9. A proposed scoring system for predicting mortality in melioidosis**

### **9.1. Introduction**

The ability to identify a high-risk group would aid in the implementation of intensive interventions, such as early antibiotic therapy, early supportive therapy and experimental immunomodulatory therapies, including G-CSF, aimed at reducing mortality. Ideally, the parameters used should be readily available to clinicians at the time of admission. In this study, a subgroup of patients presenting with acute melioidosis at highest risk of death was defined.

### **9.2. Methods**

Data on all patients presenting with culture-confirmed melioidosis to the Royal Darwin Hospital have been collected prospectively since October 1989. This data includes demographic details, details of the clinical illness and co-morbid conditions and results of radiology and laboratory findings on admission. Ethical approval to review this data was obtained from the Human Research Ethics Committee of Royal Darwin Hospital and the Menzies School of Health Research.

For the purpose of this analysis, all patients who presented with acute melioidosis confirmed by culture of *B. pseudomallei* from any site with a duration of illness less than two months were considered [1]. To preserve the assumption of independence of observations, the analysis was limited to first presentations with melioidosis, excluding relapses. Death was defined as mortality attributable to melioidosis and occurring in the context of the acute illness.

A number of clinical and simple laboratory parameters available to the clinician at the time to admission was examined (*Table 9-1*). The laboratory tests were felt to reflect dysfunction in various organ systems and associated with poor outcomes in clinical experience. Known renal impairment was defined as a creatinine of >150 µmol/L prior to the episode of melioidosis. Pneumonia was defined by clinical features suggestive of a lower respiratory tract infection with a new opacity on chest radiograph, confirmed by a positive culture of blood or sputum. Age was taken from the date of admission. If a single biochemical parameter was not assessed on

admission, it was assumed to be within the normal range; if more than one biochemical parameter was missing, the subject was excluded from the analysis.

Statistical tests were performed using Intercooled Stata 7.0 for Windows (College Station, Texas, USA). The following approach was taken; variables found to be significant on univariate logistic regression would be confirmed on multiple variable analysis using stepwise selection with a significance level of 0.2. These variables would be examined in greater detail by the use of a generalized additive model [2], performed by means of a module formulated by Royston and Ambler [3]. This non-parametric model permits examination of non-linear and threshold effects on survival by use of a scatterplot smoothing function. Breakpoints for a scoring system were based on the normal ranges of the laboratory assays, the shape of the curve on the generalized additive model and the frequency and distribution of abnormal values.

A score was formulated by the sum of the variables and plotted on a receiver-operator curve, designed to examine the relationship between sensitivity and specificity. For the purpose of this score, it was felt that sensitivity, the ability to include patients at risk of death, was of greater importance. Finally, the performance of the scoring system was assessed by examining its consistency over the various time periods.

### 9.3. Results:

During the period October, 1989 to June, 2002, 339 patients have presented to the Royal Darwin Hospital with culture-confirmed melioidosis. Fifty-one patients had chronic presentations of melioidosis and were excluded from analysis. Analyses were performed on the remaining 288 patients with acute melioidosis. In these patients median age was 49 years, 73.6% were male, and attributable mortality 25.7% (n=74). Mortality fell over time from 32.7% (35 of 107 patients) during seven wet seasons between 1989/90 and 1995/96, to 21.7% (39 of 179 patients) between 1996/97 and 2001/02 seasons.

The following factors were associated with mortality in a univariate model: pneumonia, baseline renal impairment, age on admission, serum bicarbonate, serum

urea, serum creatinine, lymphocyte count, serum bilirubin and serum sodium. Using both forward and backward stepwise selection, pneumonia, age, bicarbonate, urea, lymphocyte count and bilirubin were predictive in a multiple variable model (*Table 9-1*).

Table 9-1: Univariate and multiple variable logistic regression of predictors for mortality

<b>Categorical variables</b>				
		Deaths	Total number	Odds ratio (95% CI)
Pneumonia	Y	50	144	2.65 (1.48, 4.85)
	N	24	144	
Diabetes	Y	33	118	1.22 (0.69, 2.15)
	N	41	170	
Hazardous alcohol intake	Y	30	111	1.12 (0.63, 1.98)
	N	44	177	
Known renal impairment	Y	12	27	2.56 (1.03, 6.21)
	N	62	261	
<b>Continuous variables</b>				
		Univariate analysis (odds ratios, 95% CI)		Multiple variable analysis* (odds ratios, 95% CI)
Age		1.04 (1.02, 1.06)		1.07 (1.03, 1.10)
White cell count		1.03 (0.99, 1.07)		
Neutrophil count		1.02 (0.98, 1.07)		

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Lymphocyte count	0.47 (0.31, 0.72)	0.38 (0.20, 0.72)
Serum urea	1.05 (1.02, 1.08)	1.03 (0.99, 1.07)
Serum creatinine	1.001 (1.001, 1.003)	
Serum bilirubin	1.03 (1.01, 1.04)	1.02 (1.003, 1.04)
Serum bicarbonate	0.90 (0.86, 0.94)	0.94 (0.88, 1.01)
Serum albumin	0.96 (0.92, 1.002)	
Serum sodium	0.94 (0.90, 0.98)	
Serum potassium	1.17 (0.79, 1.74)	

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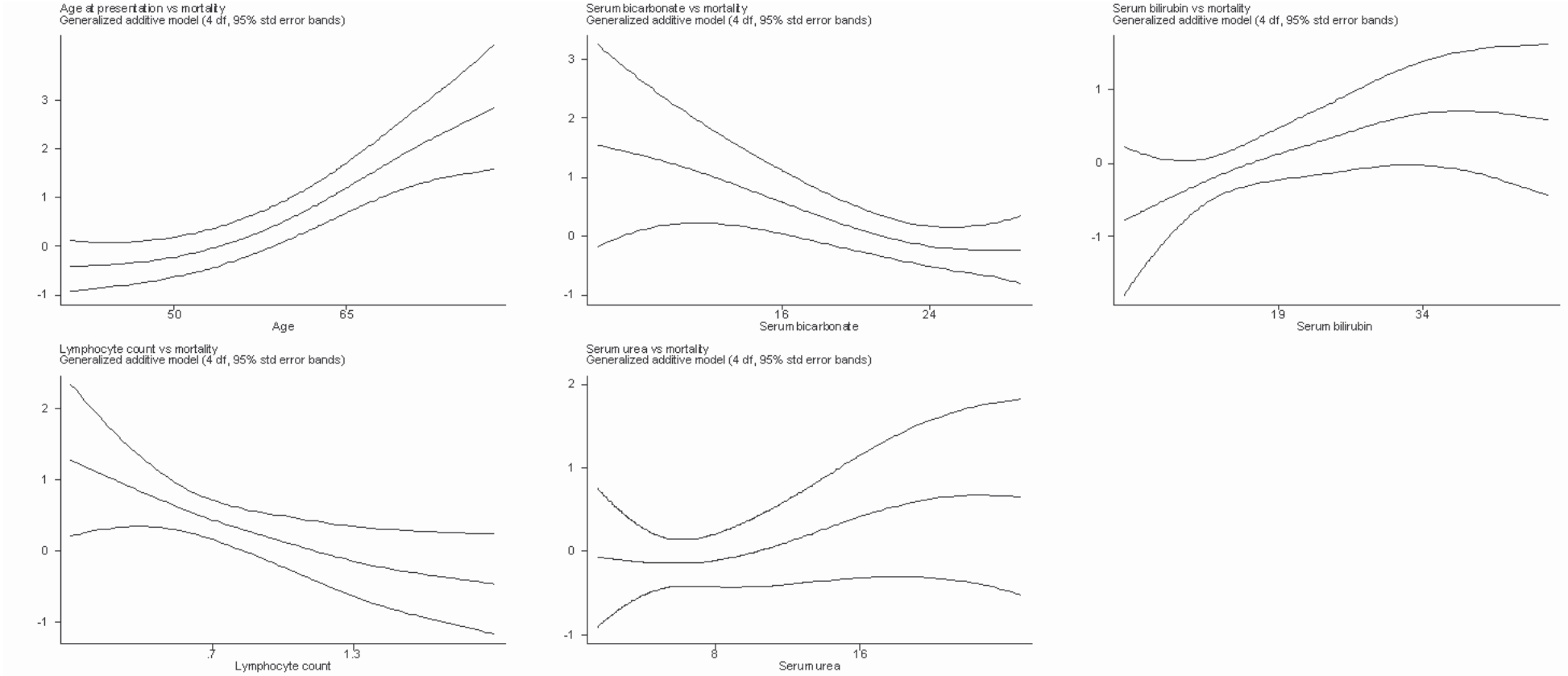
\*Multiple variable analysis included pneumonia (OR 2.54, 95% CI 1.15, 5.61)

The relationship of these variables to mortality is illustrated in Figure 9-1, with the breakpoints used for the scoring system detailed in Table 9-2. In formulating the scoring system, 27 patients, with a single biochemical parameter not assessed on admission were included in the analysis; 36 patients with more than one biochemical parameter not assessed on admission were excluded.

Table 9-2: Melioidosis score worksheet

	0	+1	+2	Component scores
Pneumonia	Absent	Present		_____
Age (years)	50 or less	51 to 64 years	65 years or older	_____
Serum bicarbonate (mmol/L)	24.1 or more	16.1-24	16 or less	_____
Serum urea (mmol/L)	Urea 8.0 or less	8.0 to 16.0	16.1 or more	_____
Serum creatinine ( $\mu\text{mol/L}$ )			250 or more	
Lymphocyte count ( $\times 10^9/\text{L}$ )	1.3 or more	0.8 to 1.2	0.7 or less	_____
Serum bilirubin ( $\mu\text{mol/L}$ )	19 or less	20 to 33	34 or more	_____
<b>Total score</b> (sum of component scores, maximum score 11)				_____

Figure 9-1: Generalized additive model plots examining non-linear relationships between continuous variables and mortality



The relationship between the total score and mortality is illustrated by the receiver operator characteristic curve (illustrated in Figure 9-2). The area under this curve was 0.78, indicating fair discriminating ability. An increase in score was associated with a rise in mortality from 6.0% in patients with scores of 0 or 1, to 81.8% in patients with scores of 8 or 9. (Figure 9-3)

Figure 9-2: Receiver-operator characteristic curve, examining the sensitivity and specificity of the total score for mortality

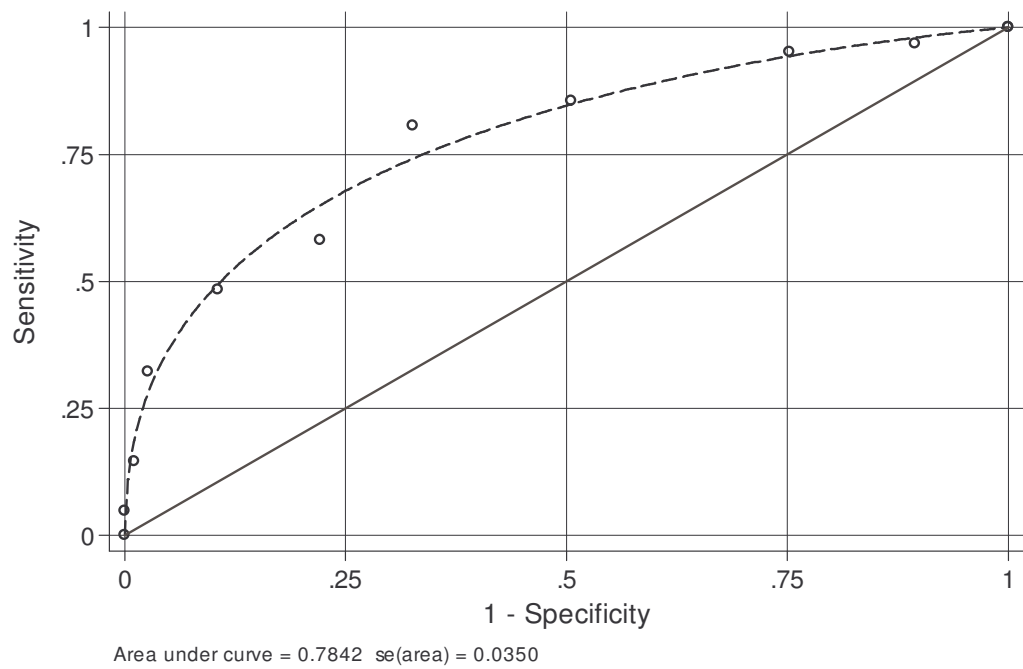
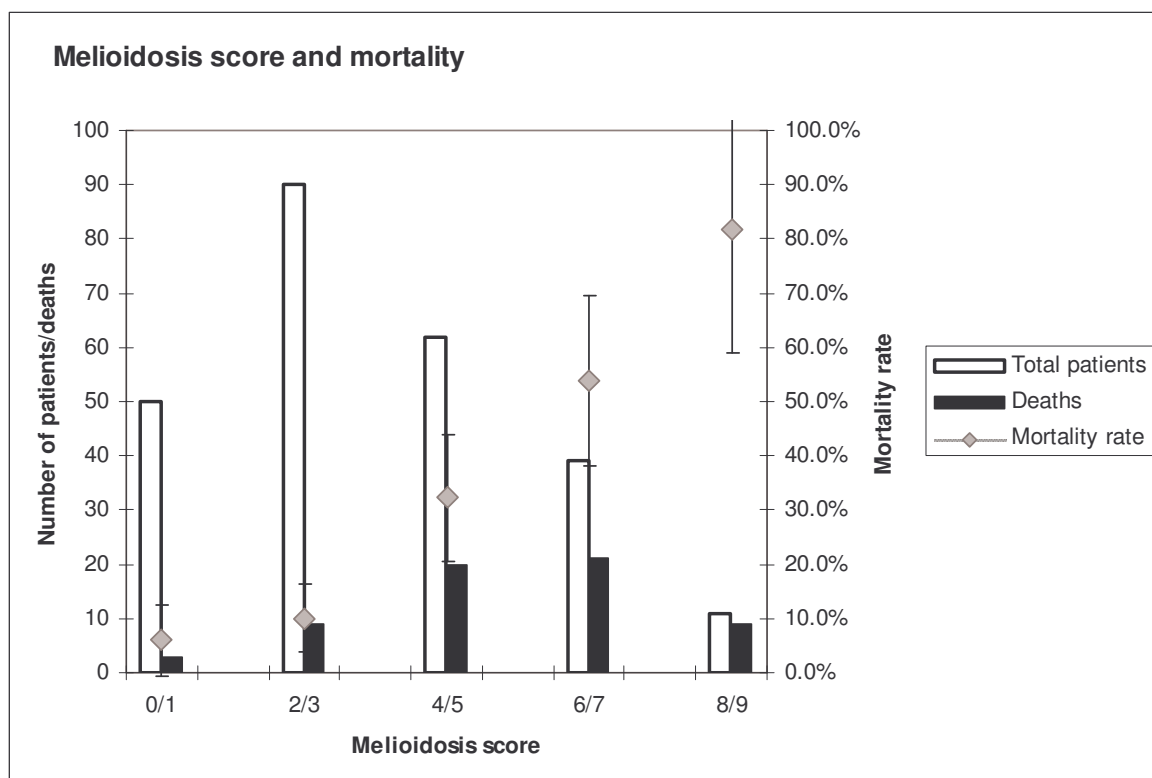


Figure 9-3: Melioidosis score; distribution of patients, deaths and mortality rate



In the group with a total score of less than or equal to 3 (n=140), there were 12 deaths with a total mortality of 8.6% (equivalent to a negative predictive value of 91.4%). Similarly, a total score of 4 or more (n=112) was associated with a mortality (equivalent to the positive predictive value) of 44.6%. At this cut-off, the sensitivity was 80.6% and specificity 67.4%, with a positive likelihood ratio of 2.5 and a negative likelihood ratio of 3.4.

The mortality in the groups with scores  $\leq 3$  and  $\geq 4$  remained relatively constant over the two periods detailed above; the positive predictive and negative predictive values during the wet seasons 1989/90 and 1995/96 was 63.9% and 87.5%, and between 1996/97 and 2001/02 35.5% and 94.1%.



#### 9.4. Discussion

The identification of risk factors in melioidosis is important for several reasons; it has implications for clinical practice, it provides an epidemiological tool to compare populations by risk and may offer clues to factors important in pathogenesis. In this observational study using data collected over 12 years, it was found that two clinical features and four biochemical parameters predicted mortality. These parameters, the presence of pneumonia, age at diagnosis, and biochemical parameters reflecting acidosis, renal dysfunction, hepatic dysfunction and lymphopaenia were used to formulate a scoring system for melioidosis.

Other parameters have been shown to be independent predictors of mortality, such as serum interleukin-6 and interleukin-10 concentrations [4, 5] and heavy bacteraemia (>50 colony forming units/mL) measured by pour plate cultures [6]. Although these assays are not performed routinely in patients with melioidosis, limiting their clinical utility, they provide important insights into pathogenesis.

Organ dysfunction has been shown to be predictive of mortality in a variety of other ICU-based scoring systems such as the Acute Physiology and Chronic Health Evaluation (APACHE), Multiple Organ Dysfunction Score (MODS), Logistic Organ Dysfunction (LOD) score, and Sequential Organ Failure Assessment (SOFA) score [7]. Although experience has shown that predicted mortality from APACHE II scores in the small number of patients with septic shock and melioidosis correlated with observed mortality (data not shown), a simpler clinical rule using routine clinical and biochemical parameters was needed. This system is designed for patients with melioidosis generally, rather than in the intensive care setting where complex ICU-based systems may be more appropriate.

Chaowagul et al have previously described similar predictors of mortality in patients with melioidosis in northeast Thailand, where a high mortality was observed in patients with low white cell counts, high urea, hypoglycaemia and liver dysfunction. However, in her series of 62 patients, limited to those with septicaemia, a detailed analysis of these factors was not attempted [8]. At the Royal Darwin Hospital, the

presence of severe sepsis, defined by standard criteria [9] has been traditionally used, as a marker of risk and the need for intensive therapies. However, although this group (n=70) had a mortality of 70%, these patients only accounted for 66% of the mortality associated with melioidosis (data not shown)

Of note, factors previously described as being predictive for the acquisition of melioidosis, such as chronic renal failure, alcoholism and diabetes [10, 11] did not independently predict mortality in this model. It is speculated that although these risk factors are important in the early pathogenesis of melioidosis, the subsequent course of the infection is best reflected by the severity of organ dysfunction. Whether this is due to bacterial factors such as inoculum dose or virulent strains, due to host factors such as patterns of cytokine responses or relative neutrophil dysfunction, or due to clinical factors such as delays in commencement of effective antibiotics and other management remains to be defined.

The association of lymphopenia with mortality is intriguing. A small group of patients with melioidosis were shown to have low lymphocyte counts in peripheral blood, but no differences were found between bacteraemic and non-bacteraemic patients. An analysis of lymphocyte subsets found depletion of T cell and NK subsets, similar to that found after administration of endotoxin [12]. As these cells are important sources of IFN- $\gamma$  in melioidosis [13] and IFN- $\gamma$  is important in resistance to *B. pseudomallei*, [14] it was speculated that these changes may provide conditions conducive to the survival and multiplication of *B. pseudomallei*.

These parameters, all available at the time of admission, have been combined in a simple predictive model. The composite melioidosis score correlates with mortality. In addition, a cut-off of 3 stratifies patients into two groups; low risk, associated with an overall mortality of less than 10%, and high-risk, associated with a mortality of over 40%. This system was validated internally by assessing its consistency over time; although there has been an overall fall in mortality during this time, mortality remained higher in the high-risk group.

There are several limitations to this study. This scoring system was derived on the basis of a relatively low number of cases and has been validated in the same dataset.

Differences in management may have implications for the generalisability of these findings; thus, this system requires prospective validation in other settings where melioidosis is commonly seen.

A Cochrane review of interventions in treating melioidosis suggested that a risk stratification system was required in considering future trials [15]; this scoring system may meet this need. This system may also allow for the identification of high risk patients who may benefit from intensive interventions aimed at reducing mortality, such as the earlier admission to intensive care, the earlier use of meropenem and supportive goal-directed resuscitation therapies [16].

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## **10. Validation of a prognostic scoring system for melioidosis**

### **10.1. Introduction**

In the previous chapter, a scoring system to stratify patients with melioidosis by mortality risk was described. Although this was validated within the constraints of the data available, external validation was required. In this study, the scoring system was validated in a distinct patient group and compared against conventional markers of clinical severity.

### **10.2. Methods**

Since 1986, a database has maintained demographic and clinical details on patients with melioidosis presenting to Sappasithiprasong Hospital, Ubon Ratchathani in northeastern Thailand. For this analysis we considered patients that presented since January 1, 1990 until January 31, 2003. We included patients that had a complete blood count, urea and electrolytes and/or liver function tests at the time of admission. A melioidosis score was calculated based on the presence of pneumonia, age, serum bicarbonate, serum bilirubin, serum urea and creatinine and lymphocyte count as previously described.

Two methods were used to handle missing data; a complete set analysis only using data from patients where all data was available, and an analysis using an imputation procedure to simulate values for the missing data based on the distribution of the existing data. In this analysis, missing values were imputed using the EM algorithm (SPSS for Windows v11.0). The EM (expectation maximization) method estimates missing values by an iterative process. Each iteration has an E step to calculate expected values of parameters and an M step to calculate maximum likelihood estimates. The EM approach assumes that the variables have a joint normal distribution [1].

The performance of the melioidosis score in patients presenting to Sappasithiprasong Hospital was tested, based on its ability to stratify patients into risk groups and its ability to distinguish patients at risk of death. A receiver operator characteristic analysis (ROC) based on the melioidosis score was performed, with a

curve fitted using a maximum likelihood technique. Finally, the cutoff derived from the ROC analysis was assessed against conventional markers of clinical severity at admission, including sepsis, septic shock and severe sepsis.

Sepsis, septic shock and severe sepsis were defined according to conventional definitions, modified slightly to adapt for the limitations of our data. Sepsis was defined as two or more of: tachycardia >90 bpm, tachypnoea >20 breaths/min or requiring mechanical ventilation, temperature >38 or <36 degrees Celsius and white cell count <4000 or >12000 cells/mL. Septic shock was defined as sepsis in association with a systolic blood pressure <90mmHg on admission or the requirement for inotropic support. Severe sepsis was defined as one of: septic shock (above), thrombocytopenia <100,000 cells/mL, bilirubin >2.0 mg/dL, creatinine >2.0 mg/dL, the requirement for ventilatory support or the finding of an unresponsive patient. Mortality was defined as a death during admission or a discharge where death was the expected outcome.

Finally direct standardization was performed, adjusted by melioidosis score, to compare the mortality rates from our previous study in Darwin, Australia to this group. Except where indicated above, statistical tests were performed using Intercooled Stata 7.0 for Windows (College Station, Texas, United States).

### **10.3. Results**

Patients during the period 1990-2002 with first presentations of culture-confirmed melioidosis where blood testing was performed on the first day of admission were considered (n=1473). Of these, 3 patients did not have an age recorded and 29 patients were lost to follow up and were excluded from further analysis. Of the remaining 1441 patients there was an overall mortality of 48.0%. Twenty patients did not have an initial assessment of creatinine, 15 did not have a baseline urea, 57 did not have bicarbonate, and 26 did not have a lymphocyte count recorded. The majority (n=1041) did not have baseline bilirubin.

#### **10.3.1. Complete set analysis**

In the 376 patients where all parameters were available, the overall mortality was 44.7%. The distribution of patients by score is illustrated in Figure 10-1; there were

no patients with scores of 1 or 0. Mortality rose with increasing score; patients with a score of 2 had a mortality of 9%, compared to patients with scores of 8 or more whose mortality was approximately 80%. The area under the ROC was 0.79 (95% CI: 0.74, 0.83; Figure 10-2). A melioidosis score of 4 or less (n=145) was associated with a mortality of 18.6%. A melioidosis score of 5 or more (n=231) was associated with a mortality of 61.0% and accounted for 83.9% of all deaths.

Figure 10-1: Melioidosis score and mortality (complete set analysis)

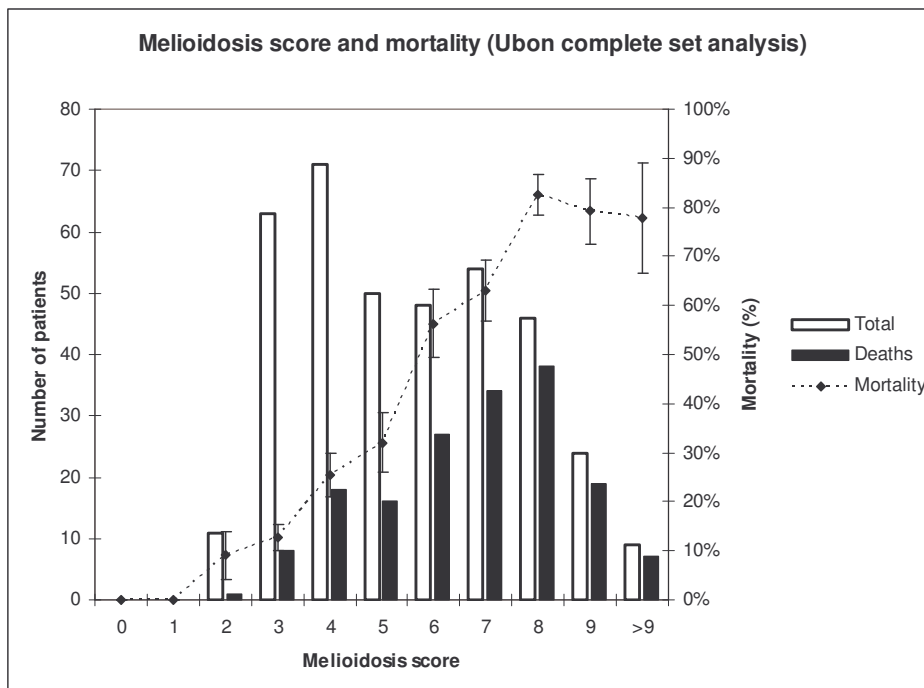
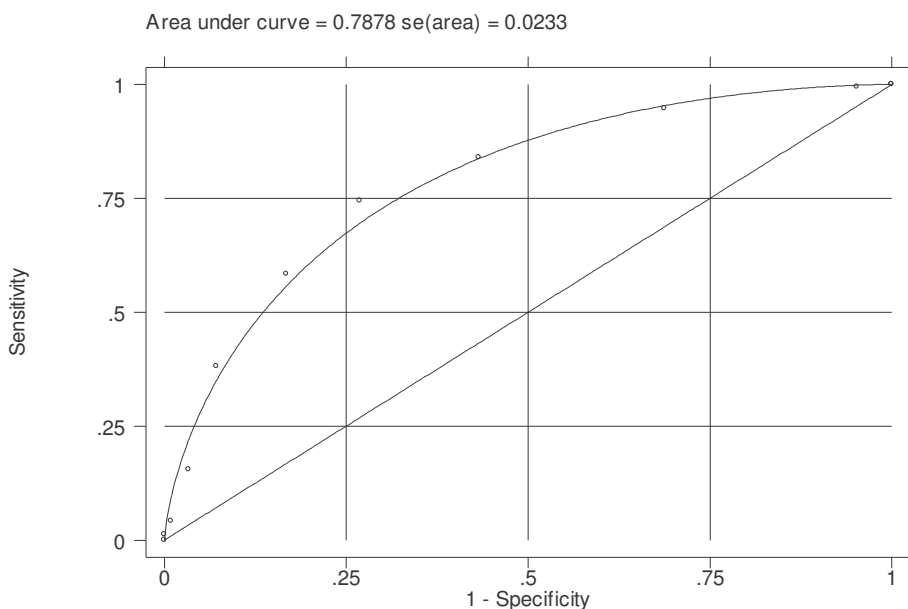


Figure 10-2: Receiver operator curve for melioidosis score vs death (complete set analysis)



### 10.3.2. Imputed values analysis

Missing values were imputed for all 1441 patients. In this analysis, mortality correlated with increasing melioidosis score with a mortality of 12% in patients with scores of 2, compared to 100% in patients with scores greater than 9 (Figure 10-3). The area under the ROC was 0.77 (95% CI: 0.74, 0.79; Figure 10-4). A melioidosis score of 4 or less was associated with a mortality of 25.6% and a score of 5 or more was associated with a mortality of 66.7% which accounted for 82.4% of all deaths.



Figure 10-3: Melioidosis score and mortality (imputed values analysis)

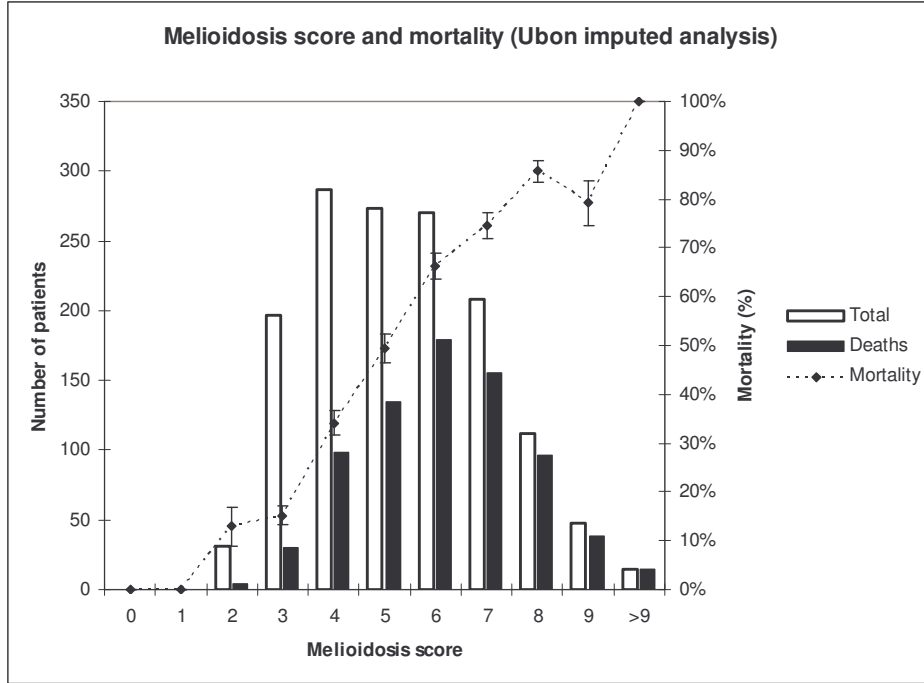
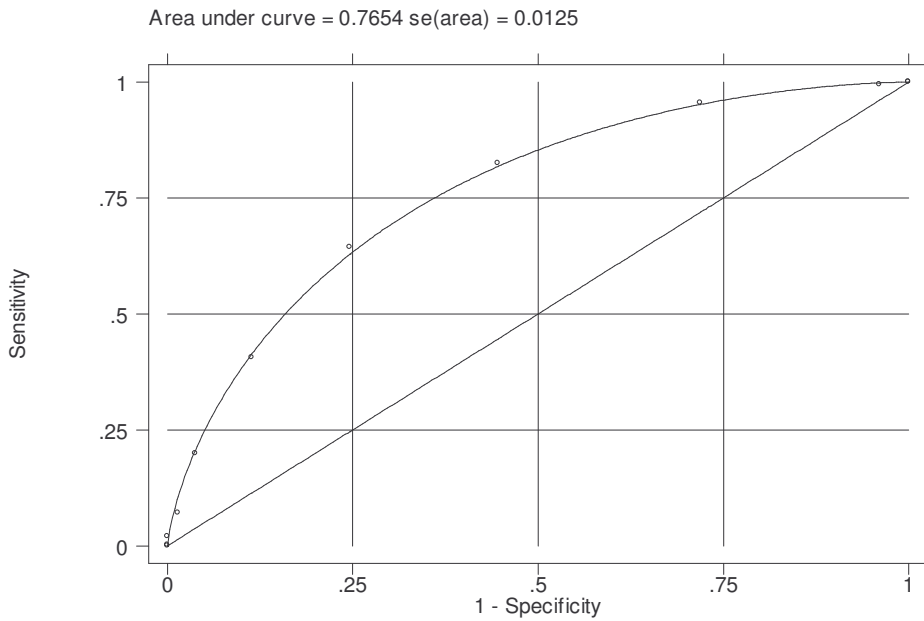


Figure 10-4: ROC curve for melioidosis score vs death (complete set analysis)



### 10.3.3. Sepsis, severe sepsis and septic shock

Presentations with sepsis were common in this group of patients (89.6%). Severe sepsis (n=964) was associated with a mortality of 68.9%; deaths in this group accounted for 84.7% of all deaths from melioidosis in this study. Septic shock (n=223) was associated with a higher mortality (84.8%) but only accounted for 25.2% of all deaths. The performance of clinical definitions and the melioidosis score in predicting mortality are summarized in Table 10-1.

*Table 10-1: Comparison of clinical definitions of sepsis severity against melioidosis score thresholds and observed mortality*

		Total	Deaths (%)	Sensitivity Specificity
<b>Clinical definitions</b>				
Sepsis	Present	1293	696 (54.0%)	92.8%
	Absent	151	54 (35.8%)	14.0%
Severe sepsis	Present	964	635 (65.9%)	84.7%
	Absent	480	115 (24.0%)	52.8%
Septic shock	Present	223	189 (84.8%)	25.2%
	Absent	1218	561 (46.1%)	95.1%
<b>Complete set analysis</b>				
Melioidosis score $\geq 4$	Present	302	159 (52.6%)	94.6%
	Absent	74	9 (12.2%)	31.3%
Melioidosis score $\geq 5$	Present	231	141 (61.4%)	83.9%
	Absent	145	27 (18.6%)	56.7%
<b>Imputed analysis</b>				
Melioidosis score $\geq 4$	Present	1213	716 (59.0%)	95.5%
	Absent	228	34 (14.9%)	28.1%
Melioidosis score $\geq 5$	Present	926	618 (66.7%)	82.4%
	Absent	515	132 (25.6%)	55.4%

#### **10.3.4. Comparison to Australian data**

Direct standardization of the mortality rate observed in this study, using the imputed values set, was performed on the 252 patients in our earlier study from Australia where melioidosis scores were able to be calculated. The standardized mortality rates for Thailand and Australia were 0.486 (95% CI: 0.464, 0.51) and 0.302 (95% CI: 0.241, 0.365) respectively which was statistically significant.

#### **10.4. Discussion**

In this study, a simple scoring system based on two clinical parameters and four biochemical and haematological parameters was validated. This system was developed from retrospective data in Darwin, Australia, but due to the relatively small numbers of patients (n=252), it was not possible to validate it in that population. The utility of such scoring systems was discussed in the previous chapter; in providing clues as to pathogenesis, as an epidemiological tool comparing patient groups and in identifying patients at risk of death. It was found that an increasing score was associated with increasing mortality, and that the sensitivity and specificity of the system in predicting death was reasonable, with an area under the ROC curve of 0.77, similar to its performance in Australia (AUC 0.78, 95% CI: 0.72, 0.85).

An unfortunate limitation of the retrospective review is that the majority of patients did not have an assessment of serum bilirubin. A complete set analysis assumes that the remaining data is representative of the missing data, and the loss of sample size limits power. Maximum likelihood imputation procedures, such as the EM algorithm, are simple and robust but are not likely to completely correct for a severe selection bias. The two methods of handling the missing data, however, yielded similar results.

Can this system be used for identifying patients at risk of death? It was found that a score of 5 or more was associated with a significantly higher mortality (>60%) than patients with lower scores, and that this group accounted for more than 80% of deaths. However, we also found that clinical definitions were equally able to stratify

patients into high and low risk groups. Patients with severe sepsis had a similar mortality rate as patients with a melioidosis score of 5 or more. Patients with septic shock had a higher mortality (>80%) but accounted for fewer of the deaths.

In contrast to Australia where blood tests are routine for all patients with melioidosis, relatively limited resources in Thailand may lead to a significant bias in testing, in that more severely ill patients are more likely to have blood tested. For example, 20% of patients in the Australian study had scores of 0 or 1, whereas no patients in the complete set analysis had similar scores. This is also reflected in the high proportion of patients with sepsis in this patient group.

Is the higher mortality in this group of patients compared to Australian patients the result of this selection bias? When standardized for the melioidosis score, the mortality rate in Ubon Ratchathani remained higher than that observed in Darwin. Thus, it is likely that differences in management, as well as than severity of illness, are important factors in the higher mortality associated with melioidosis in north eastern Thailand.

A systematic review highlighted the need for risk stratification in considering interventions for treatment of melioidosis [2]. It has been evident from previous clinical trials that different enrolment criteria have resulted in the selection of patients with different illness severity. For example, mortality in the ceftazidime-based arms of clinical trials in Thailand have varied from 14% to 47% [3-5]. Reporting of patients stratified by illness severity, using this scoring system, might allow for a comparison of such trials.

It is also evident that most deaths are attributable to the manifestations of severe sepsis, including its associated organ dysfunction. Interventions shown to be of benefit in severe sepsis include the use of intensive insulin therapy [6], goal directed resuscitation [7], activated protein C [8] and physiological dose steroids [9]. These and other adjuvant therapies in severe melioidosis may best be targeted at this group at high risk of mortality.

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## **11. Towards a clinical trial of G-CSF in melioidosis**

### **The need for a clinical trial**

The evidence supporting the use of G-CSF in melioidosis and septic shock has been discussed in chapter 3. Its use is supported by this historically controlled series, but is potentially confounded by a number of changes to management coincident with the introduction of G-CSF. However, adequately powered trials of G-CSF in non-neutropenic pneumonia and septic shock have failed to demonstrate benefit. It could be argued that on this basis, clinician uncertainty and clinical equipoise exist, making a placebo-controlled trial of G-CSF ethical. However, the failure of “patient equipoise”, discussed in chapter 6, make such a trial impossible in Darwin.

Few centres, including Darwin, admit enough patients to make such a trial viable. We considered other endemic countries, including Thailand, Malaysia and Singapore. There are few published reports of the annual numbers of patients with septic shock in these countries. Two centres were felt to have enough patients to make a trial viable; Srinagarind Hospital, Khon Kaen and Sappasithiprasong Hospital, Ubon Ratachathani, both centres with a history of clinical trials in melioidosis. We approached the Wellcome Trust-Mahidol University-Oxford University Tropical Medicine Research Program, based in Ubon Ratchathani, Thailand with a proposal to perform a clinical trial of G-CSF in melioidosis in septic shock.

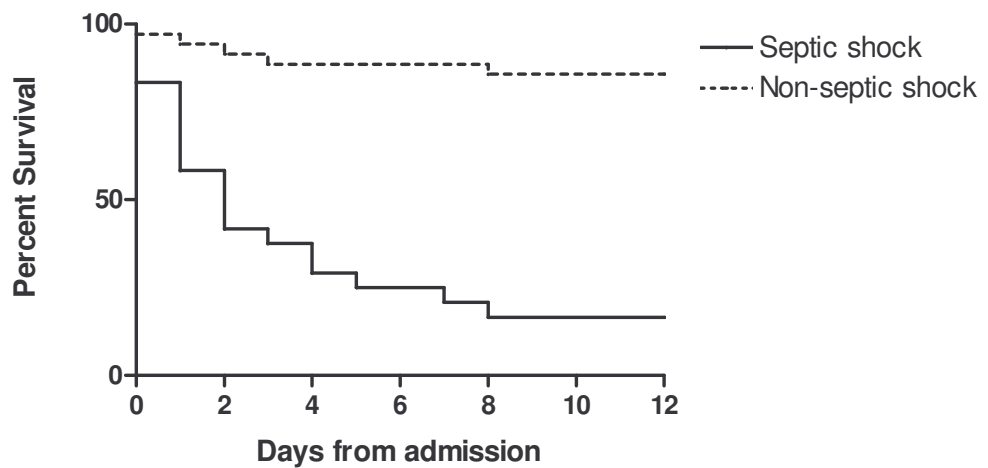
### **Barriers identified and preliminary work on feasibility**

A field trip was made in August, 2002 to the Faculty of Tropical Medicine at Mahidol University, Bangkok and to Sappasithiprasong Hospital, Ubon Ratchathani in Thailand. Concerns focussed on the cost and availability of G-CSF. It was felt that the use of G-CSF would be sustainable if a significant benefit could be demonstrated.

### ***Sample size and power:***

Previous work had demonstrated that the majority of deaths from melioidosis occurred within 48 hours [1, 2]. A review was performed of patients enrolled into

clinical trials at Ubon Ratchathani between June 1, 2002 and August 20, 2002. The enrolment criteria for the ongoing trial (ceftazidime vs ceftazidime and cotrimoxazole) was adult patients with septic melioidosis. During this time, 59 patients had culture-confirmed melioidosis with an overall mortality of 44%. Of these, 23 patients met the criteria for septic shock with a mortality of 87%. Patients with septic shock accounted for 20 of the 26 deaths (77%) observed. An analysis of time-to-death confirms that the majority of deaths occurred within the first 72 hours. It was thus felt that at the current rate of enrolment, up to 60 patients could be enrolled in a study of patients with melioidosis and septic shock within a single season.



*Figure 11-1: Survival of patients with melioidosis and septic shock in Ubon Ratchathani, 2002*

During this time, a number of differences in management were observed. These included less aggressive fluid management and lack of invasive haemodynamic monitoring, the use of mechanical ventilation outside of intensive care, the empiric use of intravenous amoxicillin-clavulanate (a preparation not available in Australia), the later presentation of patients (often referred via provincial or community hospitals), a lower nursing ratio and the less aggressive use of enteral feeding. The current standard of care was ceftazidime alone (compared to cotrimoxazole with ceftazidime or meropenem in Australia). Relevant cultural differences (specific to

the Ubon Ratchathani area) included the discharge of patients where mortality was felt to be certain, as bodies of patients who die in hospital are not permitted to return to the family house and must attend a *wat* prior to burial.

### ***Potential differential diagnoses***

A number of other infections may have resulted in diagnostic confusion, including leptospirosis, scrub typhus and other aggressive bacterial pneumonia, including *Klebsiella pneumoniae*. The use of an immunofluorescence test on direct specimens is a useful and rapid test but only helpful when positive.

A large number of patients were noted to be Cushingoid; a widespread practice is to add oral corticosteroids to herbal remedies which are available without prescription. Although steroid use may be associated with infection, we were concerned that there may have been some confusion between septic shock and Addisonian hypoadrenal crisis.

Finally, the high incidence of aplastic anaemia may have affected the efficacy of G-CSF. Neutropenic patients were to be excluded from the trial.

### ***Funding***

The manufacturers of filgrastim (Amgen, United States) and lenograstim (Chugai Pharmaceuticals, Japan) were initially approached but declined to support the trials. Approaches were made to the distributors of filgrastim in Australia (Amgen Australia) and in Thailand (Berli Jucker Pharmaceuticals and Roche Thailand) and the distributors of lenograstim in Australia (Amrad Pharmaceuticals/Merck Australia) and in Thailand (Siam Pharmaceuticals). Merck Australia agreed to donate 40 ampoules of lenograstim (Granocyte™ 263 µg) to the study.

Further funding for the purchase of lenograstim was obtained through the Murray Will Fellowship for Rural Physicians (Royal Australasian College of Physicians) and the Flinders Medical Foundation Research Grant Scheme. Partial support for airfares was obtained from the Overseas Field Trip Award and the Research Student Maintenance fund (Flinders University).



### **Proposed study**

The Thai research team had originally proposed a comparison of meropenem vs ceftazidime; as this may have been a confounding factor in a study of G-CSF vs placebo, a factorial design was proposed. The study would then be two trials conducted in parallel.

1. A study of meropenem vs ceftazidime in patients with septic melioidosis (trial A)
2. A study of G-CSF (263 mcg IV daily for 3 days) vs placebo with ceftazidime or meropenem in melioidosis with septic shock.(trial B)

Patients would be enrolled if they met standard criteria for community acquired sepsis (trial A) or septic shock (trial B), were adults >14 years and either the patient or next of kin were able to give consent. Exclusions included contraindications to the use of meropenem, ceftazidime or G-CSF or definite indications for their use (such as febrile neutropenia).

Written informed consent would be obtained by Thai-speaking study doctors or nurses. Once enrolled, patients would undergo routine blood and microbiological tests. They would be randomized into meropenem or ceftazidime (trial A) or one of four arms (trial B):

- Ceftazidime only
- Ceftazidime + G-CSF
- Meropenem only
- Meropenem + G-CSF

Doses of meropenem and ceftazidime would be adjusted according to renal function. Study drugs and treatment allocation would be concealed from clinicians and researchers. The primary endpoints would be in-hospital mortality and 28 day mortality. Secondary endpoints would be markers of morbidity and the incidence of adverse events, including organ dysfunction.

### **Sample size calculations**

In comparison of ceftazidime and meropenem (trial A) only study approximately 400 enrolled patients are needed to detect a reduction in mortality or treatment failure rate from 45% to 25% with 80% power, with the assumption that 70% of the enrolled patients will have culture-proven melioidosis. The interim analysis was to be performed after 200 patients have been enrolled (including 70 patients in G-CSF randomization)

In G-CSF study group, where we expected the mortality to be as high as 80-95%, we aimed to halve the mortality from 80% with 80% power. Thus, 56 culture-proven melioidosis cases were needed; assuming that 80% of patients had culture confirmed melioidosis, we aimed to enroll approximately 70 patients into this study.

It was assumed that an interaction did not occur between the use of meropenem and G-CSF; sample size calculations were performed for both scenarios. A sensitivity analysis was performed examining the sample size required if the mortality was 80% in the ceftazidime only group, 60% in the meropenem only and ceftazidime/G-CSF groups and 40% in the ceftazidime only group. At these levels, at 0.05 significance and 80% power, 132 patients would be required (based on a variance of proportions of 0.0200 and an average proportion of 0.600) (nQuery Advisor, Statistical Solutions, Cork, Ireland).

### **Ethical issues identified**

#### ***What about the previous evidence of efficacy?***

We have discussed ethical barriers to such a trial in Australia. However, several reasons mandate a clinical trial before G-CSF could be regarded as the standard-of-care in Thailand.

- That our previous study of G-CSF, at a single institution with a historical control, was potentially confounded by other changes to management around that time
- To assess the magnitude of benefit and thus the cost-benefit ratio
- To determine its applicability and efficacy in a Thai setting, particularly given the other differences in management to Australia

- G-CSF, although licensed in Thailand for use in febrile neutropenia, is not licensed for the treatment of melioidosis and would not be available without stronger evidence for its use.

***Would delayed consent be ethical in Thailand?***

Originally, it was proposed that delayed consent, used in studies of critically unwell patients where the efficacy of the intervention depends on its timeliness and may be affected by the consent process. This process, approved by NH&MRC guidelines for research in emergency situations, is enrolment of the patient prior to consent, with the withdrawal of the patient from the study if consent is not granted by the patient or next-of-kin.

However, following discussion with Thai researchers, it was felt that this would not be appropriate for what was perceived to be an experimental treatment.

***What about the cost of G-CSF? Would it be sustainable?***

The price of a three-day course of lenograstim compares favourably to the total cost of the currently accepted treatment, ceftazidime, and is cheaper than meropenem (Table 11-1). It was felt that if a large benefit could be demonstrated, the use of G-CSF would be sustainable. This clinically significant, sustainable difference was a 50% reduction in mortality and thus, we powered the study accordingly. It should also be noted that generic formulations of G-CSF are expected within the next few years with the expiry of the patent.

*Table 11-1: Table of relative costs: (in ThB) per day/per treatment course\**

Antibiotic	Cost per day (ThB)	Cost per treatment course (ThB)
Imipenem (1g IV tds, 10 days)	4410	44100
Meropenem (1g tds, 10 days)	4500	45000
Ceftazidime (Fortum, GlaxoSmithKline; 2g tds, 10 days)	1470	14700
Ceftazidime (Cef-4, Siam	420	4200

Pharmaceuticals; 2g tds, 10 days)		
Ampicillin-clavulanate (generic; 1.2g IV tds, 10 days)	1320	13200
Filgrastim (300mcg/day, 3 days)	4600	13800
Lenograstim (263 mcg/day, 3 days)	2500	7500

\* Pharmacy Department, Sappasithiprasong Hospital, personal communication, October 2004

The study protocol was submitted to the ethics committees at the Menzies School of Health Research and the Ministry of Public Health, Royal Government of Thailand.

No major issues were identified by the Australian reviewers. The Thai reviewers identified the following issues:

- That G-CSF was a speculative intervention unlikely to reveal benefits to patients and that the Australian data may have reflected poor management of patients with severe melioidosis in the historical control group.
- That meropenem was the best available treatment for melioidosis based on *in vitro* data demonstrating reduced endotoxin release and thus a comparison of meropenem and ceftazidime would be unethical.
- That other candidate therapies, such as IgM-enriched immunoglobulin, were more likely to be of value in melioidosis with septic shock.
- That the use of a factorial design resulted in a complex study with multiple hypotheses that would be difficult to test conclusively.

In response to this, a number of amendments were made to the study protocol to address the reviewers' concerns. The principal change was that we divided the study into two separate protocols:

- I. G-CSF vs placebo as an adjunct to ceftazidime-based standard therapy in patients with melioidosis and septic shock.
- II. Meropenem vs ceftazidime in patients with melioidosis with sepsis

Although imipenem had been tested against ceftazidime in a previous trial, and indeed had been shown to reduce endotoxin release, this was not associated with a mortality benefit in this trial [3]. Given the high cost of carbapenem antibiotics, we

argued that to justify their adoption as the standard treatment of melioidosis in Thailand a mortality benefit would have to be demonstrated in an appropriately-powered clinical trial.

It was acknowledged that there are a number of candidate immunomodulator therapies that may improve mortality from severe melioidosis, including polyclonal immunoglobulin, activated protein C and recombinant tissue factor pathway inhibitors. However, it was felt that meropenem, given its promising *in vitro* activity and reduced endotoxin release, and G-CSF, given its firm rationale and the dramatic results from Australia, were the best candidates to test in a clinical trial.

The final protocol submitted and approved by the Ministry of Public Health, Royal Government of Thailand is detailed in Appendix C

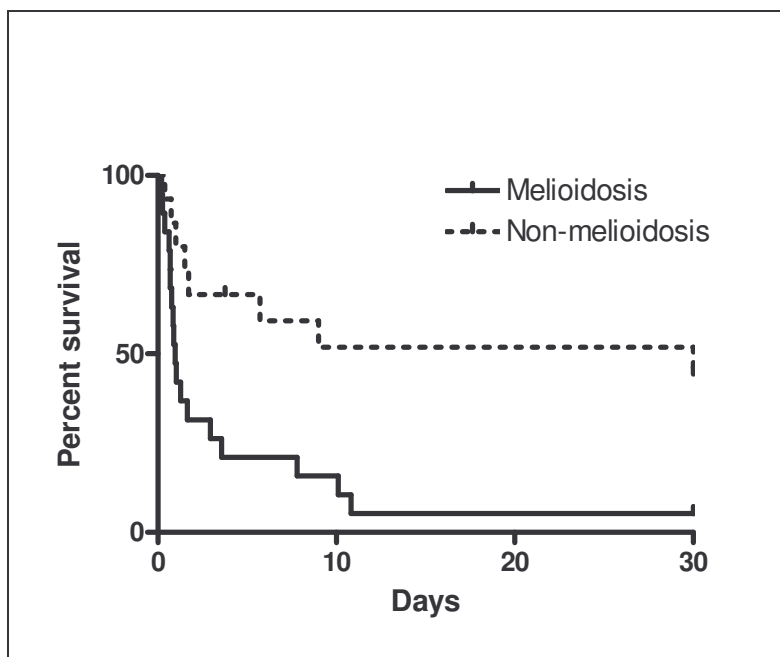
#### **Lessons learned from the trial**

This trial commenced in August 2003; patients were enrolled until November 2003 and in the subsequent season between June and November 2004. At the time of writing, 36 patients with suspected melioidosis had been enrolled, of which 22 had culture-confirmed melioidosis. An interim analysis has not yet been performed in this blinded trial, but early data is discussed here.

Difficulty was encountered in accurately identifying patients with melioidosis prior to culture results becoming available. Despite active surveillance of the wards during three times daily rounds, the severity of the illness resulted in more than half the eligible patients dying prior to enrolment. In patients with risk factors or clinical features suggesting melioidosis, immunofluorescence from sputum, urine or pus could be performed but its sensitivity was low. In many patients, melioidosis was not suspected prior to death. The fulminant nature of severe melioidosis is reflected in the high mortality of the patients that were enrolled; the Kaplan Meier survival curves are detailed in Figure 11-2. This reinforces the need for a highly sensitive and specific rapid test and/or clinical rules for the early identification of patients with melioidosis.

Important cultural differences were experienced during the study. Although it was assumed that the need for complete blinding was not required in this study using the objective endpoint of mortality, one such practice may have affected this outcome. It was noted that severely unwell patients were often discharged home against medical advice, even to the point of manual ventilation during transfer; this was attributed to cultural beliefs about dying at home, as discussed above.

Figure 11-2: Survival of patients enrolled into G-CSF trial



Although it had been appreciated that intensive care interventions were likely to be different between Australia and Thailand, the potential interaction between these and the study intervention was underestimated. Perhaps the most important of these was the nursing resources available for patient management; patients with shock and requiring ventilation were often managed on the general ward. Similarly, patients were managed by relatively inexperienced medical students and junior medical staff, albeit supported by experienced senior clinicians. Other important differences were the relative lack of sedation for intubated patients, the use of simple ventilation strategies due to the use of Bird ventilators, the lack of noradrenaline (where instead peripherally infused dopamine was used as an inotrope) and a tendency toward conservative fluid management partly due to the relative lack of renal replacement therapy and invasive monitoring. It was noted that patients enrolled in this trial manifested a severe metabolic acidosis associated with renal failure (Table 11-2) from which survival was felt to be unlikely without dialysis or haemofiltration.

*Table 11-2: Baseline data for patients enrolled in G-CSF study (n=36)*

Variable	Median	Range
Age	55 years	27-80 years
Mean arterial pressure	55 mmHg	20-106 mmHg
Glasgow coma score	13	3-15
Bilirubin	0.9 mg/dL	0.1-9.1 mg/dL
Platelets	159,000	14,000 – 500,000
PaO <sub>2</sub> /FiO <sub>2</sub>	231	67 - 713
Creatinine	4.6 mg/dL	1.1 – 20.5 mg/dL
Bicarbonate	9 mmol/L	2-28 mmol/L
pH	7.26	6.80 – 7.57
APACHE 2 score	25	15 – 43

Language and cultural barriers were largely overcome by the use of a Thai study nurse, without which this study would not have been possible. It is likely that a moderate degree of clinical acumen was lost in the translation process. The adequacy of informed consent was not formally evaluated in the study in this population; no patient declined to participate in this study. This justifies the strict review process by the Royal Government of Thailand for clinical trials.

It is anticipated that this study will continue into the coming years. It is intended that a management protocol for severely septic patients be formulated, including the possibility of increasing the availability of renal replacement therapy for severely septic patients.

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