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Adjunctive Granulocyte Colony-Stimulating Factor for Treatment of Septic Shock Due to Melioidosis

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Melioidosis, caused by the intracellular pathogen *Burkholderia pseudomallei*, is endemic in northern Australia and Southeast Asia. Risk factors for this infection have also been associated with functional neutrophil defects. Because of this, granulocyte colony-stimulating factor (G-CSF) was adopted for use in patients with septic shock due to melioidosis in December 1998. We compared the mortality rates from before and after the introduction of G-CSF therapy at the Royal Darwin Hospital (Darwin, Australia) during the period of 1989–2002. The mortality rate decreased from 95% to 10% after the introduction of G-CSF. Risk factors, the duration of illness before presentation, and the severity of illness were similar in both groups. A smaller decrease in mortality among patients in the intensive care unit who did not have melioidosis was observed, suggesting that other changes in management did not account for the magnitude of the benefit seen. We conclude that G-CSF may have contributed to the reduction in the mortality rate among patients with septic shock due to melioidosis.

Melioidosis, the infection caused by the environmental gram-negative bacillus *Burkholderia pseudomallei*, is endemic in Southeast Asia and northern Australia [1]. It is the most common bacteremic pneumonia-associated cause of death in the Top End of northern Australia [2], and, at our institution, it is the most common cause of septic shock [3].

Granulocyte colony-stimulating factor (G-CSF) is a naturally occurring cytokine that primarily increases neutrophil production. It has demonstrable effects on neutrophil function, including chemotaxis, superoxide production, and intracellular killing [4]. Other studies have demonstrated its effects on anti-inflammatory

cytokines [5] and intracellular concentrations of antibiotics [6]. Although subsequent study results were negative [7, 8], in 1998, a clinical trial was published that suggested that a subgroup of patients with severe pneumonia may benefit from the administration of recombinant human G-CSF (filgrastim) [9]. The literature about animal and human studies of the use of G-CSF for treating sepsis was examined and discussed by the intensivist and infectious diseases specialists at Royal Darwin Hospital (Darwin, Australia), and it was decided that G-CSF would be added to the means of treating septic shock in a specific attempt to reduce the almost universal mortality rate (95%) associated with septic shock due to melioidosis.

We previously reported our general experience with the use of G-CSF for treating septic shock. In that study, 6 patients in the G-CSF group and no patients in the control group had melioidosis [3]. We were unable to draw conclusions about the use of G-CSF for treating melioidosis because of the small number of cases accrued at that stage. Thus, with the benefit of further experience, we wished to audit our use of G-CSF for the treatment of melioidosis and to explore pos-

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sible confounders for the reduction in the mortality rate that we observed.

The empirical management of community-acquired sepsis in the Top End of the Northern Territory in Australia (where melioidosis is endemic) includes the use of ceftriaxone (2 g iv before transfer to the hospital) on the basis of its partial in vitro activity against *B. pseudomallei* [10] and its long half-life [11]. In the hospital, ceftriaxone is used for empirical ward management for patients who are not suspected of having melioidosis, and ceftazidime is used if melioidosis is suspected on the basis of risk factors and exposure. In January 1998, meropenem was introduced for the empirical management of community-acquired sepsis in the wet season for patients admitted to the intensive care unit (ICU). All patients admitted to the ICU with sepsis during the wet season receive meropenem as initial therapy until culture results exclude melioidosis.

METHODS

The Royal Darwin Hospital is the referral center for all patients in the Top End of Australia, which extends to north Western Australia, an area of 516,945 km² with ~150,000 inhabitants. In this area, there are only 2 urban centers with populations of >5000: Darwin (population, ~90,000) and Katherine (population, ~8000).

G-CSF therapy was adopted for use by a consensus decision of the intensivist and infectious diseases specialists at Royal Darwin Hospital in November 1998, in an attempt to reduce the almost universal rate of mortality associated with septic shock due to melioidosis seen before that time. Recombinant human G-CSF (filgrastim; Neupogen [Amgen]) was administered to all patients admitted to the ICU with septic shock, including those with melioidosis, usually within 1 h after meeting the criteria for septic shock [12]. There was no delay in administration, because a microbiological diagnosis was not required for a case to meet the criteria for treatment with G-CSF. G-CSF was administered at a dosage of 300 µg per day, given intravenously, and therapy was continued for 10 days (or longer, if the patient's condition continued to meet the definition of septic shock). The course was terminated earlier if the patient was discharged from the ICU or if the total neutrophil count was >75,000 cells/mL. Standard treatment for septic shock in the ICU at the Royal Darwin Hospital includes aggressive fluid management, vasopressor support with norepinephrine, early continuous venovenous hemofiltration for acute renal failure or severe acidosis, and early intervention with mechanical ventilation, as required.

An intensivist was first appointed in the Northern Territory in March 1998. The ICU administration before that time consisted of specialist anesthesiologists in conjunction with internists. The appointment of an intensivist resulted in many

changes to management protocols. Changes introduced in 1998 included the introduction of a closed ICU model, the use of early and aggressive enteral feeding, the use of protective ventilation strategies [13], and the more-aggressive use of hemodynamic monitoring. Other protocols were introduced during the period of 1998–2002, including a sedation protocol [14], a protocol for the use of physiological steroids for septic shock [15], and an infection-control protocol that has resulted in a decrease in the rates of nosocomial infection.

A prospective database has stored clinical details for patients with melioidosis since 1989. We included data for patients who had been admitted to the ICU during the period of August 1989 through September 2002 and who had culture-confirmed melioidosis that met the definition of septic shock. Clinical details of each case were abstracted onto standardized data forms. The use of G-CSF and possible confounding factors were analyzed for 2 time periods: December 1998 through September 2002 (patients given G-CSF; the “G-CSF group”) and before December 1998 (patients who were not given G-CSF; the “historical control group”). We defined mortality as a death that occurred during hospitalization. WBC counts were assessed on the day of ICU admission, and the highest WBC count during the subsequent 14 days was also noted.

To estimate the cumulative effect of the appointment of an intensivist and the subsequent change in ICU patient management, we examined mortality in the ICU before and after March 1998, excluding patients with culture-confirmed melioidosis. For this analysis, we examined the records for all patients admitted to the ICU during the period of 1 March 1992 through 17 July 2001. Because G-CSF was used in the treatment of patients with nonmelioidosis septic shock and has previously been reviewed [3], we considered the mortality rate among patients who did not receive a diagnosis of sepsis, pneumonia, or melioidosis—the conditions most commonly associated with septic shock.

Ethics approval for this review was obtained from the Human Research Ethical Committee of the Department of Human Services and the Menzies School of Health Research (Darwin). Statistical analysis was conducted using Intercooled Stata for Windows, version 7.0 (Stata), using Fisher's exact test and the Mann-Whitney *U* test for comparison of nonparametric data, except where indicated in the text. Statistical significance was defined as $P < .05$.

RESULTS

During the period of August 1989 through September 2002, 341 patients were admitted to the hospital with culture-confirmed melioidosis; of these patients, 42 had septic shock requiring admission to the ICU. Greater numbers of patients with septic shock were seen in 1998–1999 (9 patients) and

2000–2001 (13 patients), mostly as a result of heavy monsoonal rainfall.

During the period of December 1998 through September 2002, 21 patients were administered G-CSF for septic shock due to *B. pseudomallei* (G-CSF group), with 2 deaths (mortality rate, 9.5%). In contrast, 21 patients were admitted to the ICU during the period of August 1989 through November 1998 with septic shock due to *B. pseudomallei* (historical control group), with a single survivor (mortality rate, 95.2%; $P < .001$, by Fisher's exact test). The timing of deaths is illustrated in figure 1 (hazard ratio, 18.3; $P < .001$, by log-rank test). A summary of results is provided in table 1.

Patients had a similar median age (G-CSF group, 49 years; historical control group, 50 years; $P =$ not significant [NS]), with lower proportions of female patients (24% vs. 43%) and patients of Aboriginal ethnicity (62% vs. 81%; $P =$ NS) in the G-CSF group, although 2 patients in the G-CSF group were indigenous New Zealanders. There were no significant differences in the median durations of illness before presentation (G-CSF group, 3 days; historical control group, 4 days; $P =$ NS). Similar proportions of patients were from the urban centers of Darwin or Katherine, compared with remote communities (G-CSF group, 42%; historical control group, 38%; $P =$ NS).

Site and severity of illness. Pneumonia was the most common type of infection and was present in all but 2 patients in the G-CSF group. Ten patients (48%) in the G-CSF group and 6 patients (29%) in the historical control group had different types of infection ($P =$ NS). Risk factors were similar in both groups, with 12 patients (57%) in the historical control group and 13 (62%) in the G-CSF group having diabetes ($P =$ NS). The mean APACHE II scores were 24.8 in the G-CSF group and 25.6 in the historical control group ($P =$ NS).

Management. Use of carbapenems (imipenem or meropenem) was more common in the G-CSF group than in the historical control group (90% vs. 29%; $P < .001$). A higher proportion of patients in the historical control group received ceftriaxone as initial therapy (86% vs. 38%; $P < .01$). Three patients in the historical control group did not receive either ceftazidime or a carbapenem, because they died <48 h after admission and before receiving a diagnosis of melioidosis; all 3 patients received ceftriaxone. All patients in the G-CSF group and 15 patients in the historical control group received ceftazidime or a carbapenem before or <24 h after septic shock was diagnosed.

Safety. Of the 21 patients in the G-CSF group, 3 (14%) had WBC counts of $>75,000$ cells/mL. Three patients in the G-CSF group had electrocardiographic and/or biochemical evidence of myocardial damage; their WBC counts at this time were 10,400, 18,000, and 58,000 cells/mL. None of these cardiac events were fatal, and 1 patient had evidence of myocardial

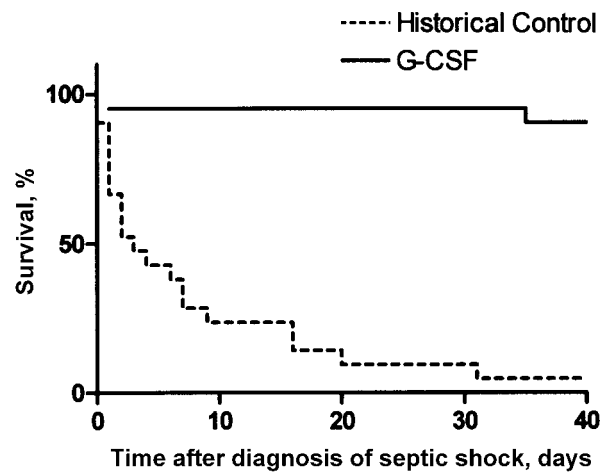


Figure 1. Kaplan Meier curve for survival of melioidosis-associated septic shock, starting from the day of hospital admission, for recipients of granulocyte colony-stimulating factor (line) and the historical control group (dotted line).

damage at presentation to the hospital. One patient in the historical control group had a myocardial infarction.

WBC counts at hospital admission were similar in both groups (median WBC count for G-CSF group, 12,400 cells/mL [range, 4900–40,200 cells/mL]; median WBC count for historical control group, 9100 cells/mL [range, 2000–36,900 cells/mL]; $P =$ NS). It was not possible to determine peak WBC counts in the historical control group because of the short duration of survival; patients in the G-CSF group had a varying increase in the WBC count (median peak WBC count, 38,500 cells/mL; range, 16,300–90,900 cells/mL) after a median of 6 days (range, 1–11 days).

Analysis of all ICU admissions and mortality. During the period of 1 March 1992 through 14 August 2001, data were available for 3147 patients admitted to the ICU, 2647 of whom did not have melioidosis, sepsis, or pneumonia. Significant diagnoses (i.e., those that occurred in $>2\%$ of patients), apart from sepsis, melioidosis, and pneumonia, were multiple trauma (9.3%) and head trauma (6.8%), chronic obstructive airway disease and asthma (5.5%), intracranial hemorrhage (5.4%), cardiac failure and cardiogenic shock (5.2%), neurological disease (4.6%), cardiac arrest (4.2%), gastrointestinal perforation and obstruction (3.1%), drug overdose (2.7%), and seizures (2.3%).

In the period before 1 March 1998, the mean APACHE II score for 1669 patients was 16.6, and the observed mortality rate was 23.7%. In the period starting on 1 March 1998, the mean APACHE II score for 978 patients was 17.8, and the observed mortality rate was 21.3%. In a Poisson regression comparison of the period starting on 1 March 1998 with the

Table 1. Summary of results of a study of adjunctive granulocyte colony-stimulating factor (G-CSF) for treatment of septic shock due to melioidosis

Characteristic or finding	G-CSF recipients (n = 21)	Historical control subjects (n = 21)	P
Mortality	2 (9.5)	20 (95.2)	<.001
Australian Aboriginal race	13 (62)	17 (81)	NS
Age, median years (range)	49 (28–64)	50 (11–74)	NS
Male sex	16 (76)	12 (57)	NS
Duration of illness before presentation, median days (range)	3 (1–8)	4 (1–90)	NS
Patient from urban setting	9 (42)	8 (38)	NS
Pneumonia present	19 (90)	21 (100)	NS
Other type of infection present	10 (48)	6 (28)	NS
Diabetes	13 (57)	12 (62)	NS
Mean APACHE II score (range)	24.8 (13–33)	25.6 (13–44)	NS
Receipt of carbapenem	19 (90)	6 (28)	<.001
Time to receipt of ceftazidime or carbapenem, median days (range) ^a	0 (–15 to 0)	0 (–2 to 5)	.007
Use of ceftriaxone as initial therapy	8 (38)	18 (86)	.004
Time to receipt of ceftriaxone, ceftazidime, or carbapenem, median days (range) ^a	0 (–15 to 0)	0 (–5 to 2)	NS
WBC count, median cells/mL (range)			
At admission to the ICU	12,400 (4900–40,200)	9100 (2000–36,900)	NS
Peak	38,500 (16,300–90,900)	Not analyzed	...

NOTE. Data are no. (%) of patients, unless otherwise indicated. ICU, intensive care unit; NS, not significant.

^a Time was relative to day of diagnosis of septic shock (negative values indicate that the antibiotic was received before the diagnosis of septic shock). Four patients in the historical control group did not receive ceftazidime or carbapenem.

period before that date, the APACHE II–adjusted mortality ratio was 0.77 ($P < .002$).

DISCUSSION

The rationale for introducing G-CSF for the treatment of septic shock due to melioidosis was based on the following data available at the time: *B. pseudomallei* has been shown to survive and multiply within cells, including neutrophils [16]; comorbid conditions associated with mortality due to and development of melioidosis [2], including diabetes, chronic renal failure, and hazardous alcohol use, are also associated with functional neutrophil defects [17–19]; G-CSF has been shown to improve the outcomes of sepsis in animal models and to improve neutrophil function in vitro [19, 20]; evidence available at that time suggested that G-CSF therapy may benefit patients with multilobar pneumonia—although no benefit was seen overall [9]—and patients also had improvements in diabetic foot ulcers associated with increased neutrophil function [21]; and G-CSF is generally well tolerated, with an extensive history of use for the treatment of neutropenia [4].

Our subsequent experience, with an associated reduction in the mortality rate for this condition from 95% to 10%, has

been in sharp contrast to the findings of large, published studies of G-CSF for treatment of nonneutropenic infection. In recent studies, no benefit was attributed to the use of G-CSF in patients with community-acquired multilobar pneumonia [7] or severe pneumonia and severe sepsis [8, 22]. In the study by Root et al. [8], investigators suggested that delays in administering G-CSF might have contributed to the negative results. We have always administered G-CSF very shortly after admission to the ICU and after the diagnosis of septic shock.

In performing this retrospective review of our experience with G-CSF, we considered several potential confounders, but we believe that each of them would be unlikely to result in such a large reduction in the mortality rate. Could the appointment of an intensivist have reduced mortality to this extent? Clearly, the appointment of an intensivist has been associated with a modest improvement in the mortality rate at our institution, with a decrease in the rate of mortality due to septic shock (for which mortality is confounded by routine G-CSF use) [3] and in critically ill patients with other diagnoses. Although other studies examining the effect of intensivists on mortality are likely to suffer from a significant publication bias, the magnitude of the effect at Royal Darwin Hospital (a 36% reduction in the mortality rate, with adjustment for severity of

illness) is in line with the findings of published studies [23–26]. Thus, it is unlikely that this factor is entirely responsible for the 90% reduction in mortality among patients with melioidosis-associated septic shock.

Could an earlier diagnosis and earlier administration of antibiotics have resulted in this effect? Ceftriaxone was used more often as initial therapy in the historical control group, and the G-CSF group received ceftazidime or a carbapenem antibiotic earlier. A retrospective review from Thailand suggested that there were poorer outcomes in patients treated initially with ceftriaxone or cefotaxime than in those treated with ceftazidime or carbapenems [27]. However, that study did not control for time to receipt of antibiotics or severity of illness, and patients treated with a third-generation cephalosporin were more likely to be bacteremic (78% vs. 62%). Patients treated empirically with ceftazidime or a carbapenem had a lower mortality rate than did those who were treated with a third-generation cephalosporin and whose therapy was changed to ceftazidime or a carbapenem (42% vs. 61%). This difference, a relative rate reduction of 31%, is unlikely to fully account for the difference in mortality seen in our series, although we acknowledge that it potentially represents a significant confounding factor. In the absence of data from clinical trials, we continue to advocate the use of ceftriaxone (2 g iv, which is greater than the conventional dose used in Thailand, 20 mg/kg iv) for the empirical management of adult community-acquired sepsis in our region. Ceftriaxone has in vitro activity against *B. pseudomallei* and other common bacteria that cause community-acquired sepsis, it is available in remote settings, and it has a long half-life, which is important when considering delays in medical evacuation from remote settings [10, 11].

Delays in treatment due to transport may have impacted on the course of the illness, but we did not find any differences in the geographical locations of patients in the 2 groups. Such delays before admission would be expected to result in more severely unwell patients, and we did not find any significant differences between groups in the severity of illness at admission to the ICU.

Could other changes in management have accounted for this effect? Although meropenem has yet to be tested in a clinical trial, another carbapenem antibiotic, imipenem, was tested in Thailand [28]. Although that trial was underpowered as a result of withdrawal of funding, no difference in mortality was seen between groups after enrolment of 214 patients with culture-confirmed melioidosis (the overall mortality rate was 36.9%). This suggests that, if such a difference exists, it would likely be small. With regard to the other changes made to management protocols around this time, only protective ventilation strategies [13] and the use of aggressive monitoring with early goal-

directed resuscitation [29] have been demonstrated to have an impact on mortality.

Could changes in admission criteria for the ICU have selected for patients who were more likely to survive? There have not been any changes to ICU admission policies with respect to patient selection during this time. We found that the G-CSF and historical control groups had similar severities of illness, as measured by APACHE II scores. Every patient in this study with melioidosis had the presence of comorbidities recognized as a risk factor for melioidosis.

Are other clinical features and in vivo models consistent with a beneficial effect? Such features may have included a quicker resolution of fever and shorter duration of blood culture positivity; however, such comparisons would not be meaningful as a result of the short duration of survival of patients before G-CSF began to be used. A research group recently studied G-CSF as an adjunct to ceftazidime in a Balb/c mouse model of acute melioidosis [30]. In these studies, there was no benefit associated with the use of G-CSF with ceftazidime. However, given that findings from previous animal studies using other pathogens have failed to translate to humans in clinical trials, such animal models may not be indicative of benefits of this therapy for humans.

A previous concern has been that G-CSF use may increase the incidence and severity of sepsis-induced acute respiratory distress syndrome (ARDS) [31]. We found that we could not readily retrospectively identify ARDS in patients with pneumonia, but in all 4 clinical trials of G-CSF for pneumonia [7–9, 22], there was not a significant increase in the incidence of ARDS, organ dysfunction, or serious adverse events.

Within the limitations of the study design, we observed a decrease in the mortality rate from 95% to 10%, which was associated with the use of G-CSF. Although we cannot exclude the possibility that this may have resulted from a convergence of confounding factors, including the presence of an intensivist and the earlier use of effective antibiotics, the decrease in mortality is in excess of that which might be ascribed to these factors. It is hypothesized that the prompt use of G-CSF in our patients with comorbid conditions associated with neutrophil dysfunction may have contributed, at least in part, to the reduction in mortality associated with the intracellular pathogen *B. pseudomallei*. These results deserve further scrutiny; in conjunction with colleagues in Thailand, we are planning a placebo-controlled, randomized, controlled trial.

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References

1. Dance DAB. Melioidosis: the tip of the iceberg? *Clin Microbiol Rev* **1991**; 4:52–60.
2. Currie BJ, Fisher DA, Howard DM, et al. Endemic melioidosis in tropical northern Australia: a 10-year prospective study and review of the literature. *Clin Infect Dis* **2000**; 31:981–6.
3. Stephens DP, Fisher DA, Currie BJ. An audit of the use of granulocyte colony-stimulating factor in septic shock. *Intern Med J* **2002**; 32:143–8.
4. Welte K, Gabrilove J, Bronchud MH, Platzer E, Morstyn G. Filgrastim (r-metHuG-CSF): the first 10 years. *Blood* **1996**; 88:1907–29.
5. Weiss M, Moldawer LL, Schneider EM. Granulocyte colony-stimulating factor to prevent the progression of systemic nonresponsiveness in systemic inflammatory response syndrome and sepsis. *Blood* **1999**; 93: 425–39.
6. McKenna P, Nelson S, Andresen J. Filgrastim (rHuG-CSF) enhances ciprofloxacin uptake and bactericidal activity of human neutrophils in vitro [abstract 535]. *Am J Respir Crit Care Med* **1996**; 153:A535.
7. Nelson S, Heyder AM, Stone J, et al. A randomized controlled trial of filgrastim for the treatment of hospitalized patients with multilobar pneumonia. *J Infect Dis* **2000**; 182:970–3.
8. Root RK, Lodato RF, Patrick W, et al. Multicenter, double-blind, placebo-controlled study of the use of filgrastim in patients hospitalized with pneumonia and severe sepsis. *Crit Care Med* **2003**; 31:367–73.
9. Nelson S, Belknap SM, Carlson RW, et al. A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. CAP Study Group. *J Infect Dis* **1998**; 178:1075–80.
10. Jenney AW, Lum G, Fisher DA, Currie BJ. Antibiotic susceptibility of *Burkholderia pseudomallei* from tropical northern Australia and implications for therapy of melioidosis. *Int J Antimicrob Agents* **2001**; 17:109–13.
11. Currie B, Fisher D, Anstey N, Lum G. Antibiotic protocol for adult community acquired pneumonia in the Top End. The Northern Territory Disease Control Bulletin **2000**; 7:5–6.
12. ACCP/SCCM. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* **1992**; 20:864–74.
13. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. *N Engl J Med* **2000**; 342:1301–8.
14. Brook AD, Ahrens TS, Schaiff R, et al. Effect of a nursing-implemented sedation protocol on the duration of mechanical ventilation. *Crit Care Med* **1999**; 27:2609–15.
15. Annane D, Sebille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* **2002**; 288:862–71.
16. Jones AL, Beveridge TJ, Woods DE. Intracellular survival of *Burkholderia pseudomallei*. *Infect Immun* **1996**; 64:782–90.
17. Marhoffer W, Stein M, Schleinkofer L, Federlin K. Evidence of ex vivo and in vitro impaired neutrophil oxidative burst and phagocytic capacity in type 1 diabetes mellitus. *Diabetes Res Clin Pract* **1993**; 19: 183–8.
18. Salant DJ, Glover AM, Anderson R, et al. Depressed neutrophil chemotaxis in patients with chronic renal failure and after renal transplantation. *J Lab Clin Med* **1976**; 88:536–45.
19. Nelson S, Summer W, Bagby G, et al. Granulocyte colony-stimulating factor enhances pulmonary host defenses in normal and ethanol-treated rats. *J Infect Dis* **1991**; 164:901–6.
20. Serushago BA, Yoshikai Y, Handa T, Mitsuyama M, Muramori K, Nomoto K. Effect of recombinant human granulocyte colony-stimulating factor (rh G-CSF) on murine resistance against *Listeria monocytogenes*. *Immunology* **1992**; 75:475–80.
21. Gough A, Clapperton M, Rolando N, Foster AV, Philpott-Howard J, Edmonds ME. Randomised placebo-controlled trial of granulocyte-colony stimulating factor in diabetic foot infection. *Lancet* **1997**; 350: 855–9.
22. Wunderink RG, Leeper KV, Schein R, et al. Filgrastim in patients with pneumonia and severe sepsis or septic shock. *Chest* **2001**; 119:523–9.
23. Reynolds HN, Haupt MT, Thill-Baharozian MC, Carlson RW. Impact of critical care physician staffing on patients with septic shock in a university hospital medical intensive care unit. *JAMA* **1988**; 260: 3446–50.
24. Multz AS, Chalfin DB, Samson IM, et al. A “closed” medical intensive care unit (MICU) improves resource utilization when compared with an “open” MICU. *Am J Respir Crit Care Med* **1998**; 157:1468–73.
25. Ghorra S, Reinert SE, Cioffi W, Buczko G, Simms HH. Analysis of the effect of conversion from open to closed surgical/par intensive care unit. *Ann Surg* **1999**; 229:163–71.
26. Blunt MC, Burchett KR. Out-of-hours consultant cover and case-mix-adjusted mortality in intensive care. *Lancet* **2000**; 356:735–6.
27. Chaowagul W, Simpson AJ, Suputtamongkol Y, White NJ. Empirical cephalosporin treatment of melioidosis. *Clin Infect Dis* **1999**; 28:1328.
28. Simpson AJ, Suputtamongkol Y, Smith MD, et al. Comparison of imipenem and ceftazidime as therapy for severe melioidosis. *Clin Infect Dis* **1999**; 29:381–7.
29. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* **2001**; 345:1368–77.
30. Powell K, Ulett G, Hirst R, Norton R. G-CSF immunotherapy for treatment of acute disseminated murine melioidosis. *FEMS Microbiol Lett* **2003**; 224:315–8.
31. Azoulay E, Attalah H, Harf A, Schlemmer B, Delclaux C. Granulocyte colony-stimulating factor or neutrophil-induced pulmonary toxicity: myth or reality? Systematic review of clinical case reports and experimental data. *Chest* **2001**; 120:1695–701.