MAJOR ARTICLE

DALI: Defining Antibiotic Levels in Intensive Care Unit Patients: Are Current β-Lactam Antibiotic Doses Sufficient for Critically Ill Patients?

Jason A. Roberts,[1,2] Sanjoy K. Paul,[3,4] Murat Akova,[5] Matteo Bassetti,[6] Jan J. De Waele,[7] George Dimopoulos,[8] Kirsi-Maija Kaukonen,[9] Despoina Koulenti,[1,8] Claude Martin,[10,11] Philippe Montravers,[12] Jordi Rello,[13] Andrew Rhodes,[14] Therese Starr,[2] Steven C. Wallis,[1] and Jeffrey Lipman[1,2]; for the DALI Study[a]

[1]Burns Trauma and Critical Care Research Centre, University of Queensland, [2]Royal Brisbane and Women's Hospital, [3]Clinical Trials and Biostatistics Unit, QIMR Berghofer Medical Research Institute, and [4]Queensland Clinical Trials and Biostatistics Centre, School of Population Health, University of Queensland, Brisbane, Australia; [5]School of Medicine, Hacettepe University, Ankara, Turkey; [6]Azienda Ospedaliera Universitaria Santa Maria della Misericordia, Udine, Italy; [7]Ghent University Hospital, Ghent, Belgium; [8]Attikon University Hospital, Athens, Greece; [9]Helsinki University Central Hospital, Helsinki, Finland; [10]Hospital Nord, Marseille, [11]AzuRea Group, and [12]Centre Hospitalier Universitaire Bichat-Claude Bernard, AP-HP, Université Paris VII, France; [13]CIBERES, Vall d'Hebron Institut of Research, Universitat Autonoma de Barcelona, Spain; and [14]St George's Healthcare NHS Trust and St George's University of London, United Kingdom

(See the Editorial Commentary by Craig on pages 1084–5.)

Background. Morbidity and mortality for critically ill patients with infections remains a global healthcare problem. We aimed to determine whether β-lactam antibiotic dosing in critically ill patients achieves concentrations associated with maximal activity and whether antibiotic concentrations affect patient outcome.

Methods. This was a prospective, multinational pharmacokinetic point-prevalence study including 8 β-lactam antibiotics. Two blood samples were taken from each patient during a single dosing interval. The primary pharmacokinetic/pharmacodynamic targets were free antibiotic concentrations above the minimum inhibitory concentration (MIC) of the pathogen at both 50% (50% $fT_{>MIC}$) and 100% (100% $fT_{>MIC}$) of the dosing interval. We used skewed logistic regression to describe the effect of antibiotic exposure on patient outcome.

Results. We included 384 patients (361 evaluable patients) across 68 hospitals. The median age was 61 (interquartile range [IQR], 48–73) years, the median Acute Physiology and Chronic Health Evaluation II score was 18 (IQR, 14–24), and 65% of patients were male. Of the 248 patients treated for infection, 16% did not achieve 50% $fT_{>MIC}$ and these patients were 32% less likely to have a positive clinical outcome (odds ratio [OR], 0.68; $P = .009$). Positive clinical outcome was associated with increasing 50% $fT_{>MIC}$ and 100% $fT_{>MIC}$ ratios (OR, 1.02 and 1.56, respectively; $P < .03$), with significant interaction with sickness severity status.

Conclusions. Infected critically ill patients may have adverse outcomes as a result of inadeqaute antibiotic exposure; a paradigm change to more personalized antibiotic dosing may be necessary to improve outcomes for these most seriously ill patients.

Keywords. continuous infusion; extended infusion; adverse events; pharmacokinetics; pharmacodynamics.

Infections in critically ill patients are a major burden to the healthcare system. Of concern for clinicians and administrators, neither the incidence of these infections over the past 30 years nor the mortality rates appear to be improving. This challenging dilemma has led to 70% of all intensive care unit (ICU) patients being

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[a]All members of the DALI Study Group are listed in the Appendix.

Correspondence: Jason A. Roberts, PhD, Burns Trauma and Critical Care Research Centre, University of Queensland, Level 3 Ned Hanlon Bldg, Royal Brisbane and Women's Hospital, Butterfield St, Brisbane 4029, Queensland, Australia (j.roberts2@uq.edu.au).

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prescribed antibiotics at any one time [1]. With such high rates of usage, it is easy to understand why the ICU stay is associated with the development of increasing levels of antibiotic-resistant bacteria that then pervade other healthcare settings.

For severe infections causing sepsis and septic shock, the early initiation of antibiotics with an appropriate spectrum for the likely pathogen has been demonstrated to be an effective intervention [2–4]. It has been suggested that superior infection outcomes could be achieved in critically ill patients by optimization of the pharmacokinetic exposure of antibiotics [5, 6]. These suggestions are based, in part, on numerous data demonstrating grossly altered pharmacokinetics in critically ill patients from small single-center studies [7]. Given that antibiotic dosing regimens are derived from healthy volunteers and do not account for these major differences in drug disposition, the present approach is likely to lead to suboptimal outcomes for critically ill patients [5, 8].

The β-lactams (penicillins, cephalosporins, carbapenems, and monobactams) are the most commonly prescribed family of antibiotics. From a pharmacokinetic/pharmacodynamic (PK/PD) perspective, preclinical studies have defined these antibiotics to be time dependent, that is, the time for which the free (unbound) antibiotic concentration is maintained above the minimum inhibitory concentration (MIC) is the determinate factor associated with bacterial killing ($fT_{>MIC}$) [9, 10]. Whereas animal in vivo studies have defined an $fT_{>MIC}$ between 40% and 70% of the dosing interval as being necessary [11], retrospective clinical evaluations have suggested that larger drug exposures are required, with β-lactam concentrations up to 4 times the MIC for the entire dosing interval being suggested [12, 13]. However, it remains unclear what PK/PD exposure is clinically necessary for maximal patient benefit.

With the present level of knowledge, there are few robust data to direct further improvement for antibiotic treatment in critically ill patients. Limiting progress is the absence of large-scale data on the appropriateness of present dosing. To address these deficiencies, we undertook the DALI (Defining Antibiotic Levels in Intensive Care Patients) study.

The primary objective of the study was to determine whether contemporary β-lactam antibiotic dosing in critically ill patients across a large number of ICUs achieves concentrations associated with maximal activity. The secondary objective was to correlate the observed antibiotic PK/PD with the clinical outcomes of therapy.

METHODS

The DALI study was a prospective, multicenter pharmacokinetic point-prevalence study. The detailed protocol for this study has been published previously [14]. The β-lactam antibiotics eligible for this analysis were amoxicillin (coadministered with clavulanate), ampicillin, cefazolin, cefepime, ceftriaxone, doripenem, meropenem, and piperacillin (coadministered with tazobactam).

Ethical approval to participate in this study was obtained at all participating centers and informed consent was obtained for each patient. The lead site was the University of Queensland, Australia (approval number 201100283, May 2011). Patients were all identified for participation by clinical ICU staff on the Monday of the nominated sampling week, with blood sampling and data collection occurring throughout that week.

PK/PD Targets

The PK/PD ratio is defined as the ratio between the measured free antibiotic concentration in plasma at 50% or 100% of the dosing interval and the MIC. The target PK/PD ratios used in this study are shown in Table 1. Where available, the MIC of the known pathogen was provided by the local microbiology laboratory. Where an MIC was not available, as many centers do not routinely generate these data, the MIC of the pathogen was defined by the European Committee on Antimicrobial Susceptibility Testing's MIC_{90} data (http://www.eucast.org/clinical_breakpoints). Where no pathogen was formally identified, the highest MIC for susceptible bacteria to the antibiotic was assumed. These breakpoints were chosen for a worst-case scenario of bacterial susceptibility, which is what empiric dosing is based upon.

Table 1. Definitions Used for Pharmacokinetic/Pharmacodynamic and Clinical Endpoints

PK/PD Target	Description
50% $fT_{>MIC}$	Free drug concentration maintained above MIC of the known or suspected pathogen for at least 50% of dosing interval. This was considered to be the most conservative PK/PD target.
50% $fT_{>4\times MIC}$	Free drug concentration maintained above a concentration 4-fold higher than the MIC of the known or suspected pathogen for at least 50% of dosing interval.
100% $fT_{>MIC}$	Free drug concentration maintained above MIC of the known or suspected pathogen throughout the entire dosing interval.
100% $fT_{>4\times MIC}$	Free drug concentration maintained above a concentration 4-fold higher than the MIC of the known or suspected pathogen throughout the entire dosing interval.
Positive clinical outcome	Completion of treatment course without change or addition of antibiotic therapy, and with no additional antibiotics commenced with 48 h of cessation. De-escalation to a narrower spectrum antibiotic was permitted but excluded from the clinical outcome analysis.
Negative clinical outcome	Any clinical outcome other than positive clinical outcome.

Abbreviations: MIC, minimum inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic.

Study Treatments and Blood Sampling

Antibiotic dosing was done as per the treating clinician and therapy could be administered by either intravenous intermittent or continuous infusion. Each patient had 2 blood samples taken for each β-lactam antibiotic he or she was receiving. Blood sample A was a mid-dose blood sample at 50% of the way through a dosing interval and blood sample B was a predose level at the end of a dosing interval. The observed concentrations were then interpreted in relation to the known or presumed MIC of the pathogen. For example, the 100% $fT_{>4\times MIC}$ would be attained if the blood sample B concentration exceeded the MIC by at least a factor of 4.

Data Collection

Data collection was performed by trained staff at each participating center and entered onto a case report form. Various demographic and clinical data were collected including age, sex, height, weight, presence of renal replacement therapy, and measures of organ function and levels of patient sickness severity as described by the APACHE II (Acute Physiology and Chronic Health Evaluation II) score [15] on admission and SOFA (Sequential Organ Failure Assessment) score on day of sampling [16]. Mortality at 30 days was also collected. Clinical outcome of therapy was assessed using the definitions in Table 1. Combination therapy was defined as the concomitant use of ≥2 antibiotics of different mechanistic classes at the time of pharmacokinetic sampling.

Antibiotic dosing data including the dose, infusion duration, frequency of administration, the time of dosing and sampling, and the day of antibiotic therapy were collected. All data were collated by the coordinating center (Burns Trauma and Critical Care Research Centre, University of Queensland, Australia).

Maintenance of Sample Integrity

Blood samples were processed and stored per protocol to maintain integrity. A commercial courier company transported the clinical samples on dry ice to the coordinating center.

Bioanalysis

The concentration of the study antibiotics in the biological samples were determined by validated chromatographic methods (high performance liquid chromatography and liquid chromatography with tandem mass spectrometry) (US Food and Drug Administration guidelines: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf). Unbound drug concentrations were directly measured for the highly protein-bound drugs cefazolin and ceftriaxone using ultrafiltration with 30 kDa cutoff devices (Centrifree, Merck Millipore, Tullagreen, Ireland) [17].

Statistical Analysis

Basic statistics on demographic, clinical, and PK/PD-related data were presented by number (%) or median (interquartile range [IQR]), as appropriate. The distributions of clinical and PK/PD-related study parameters were compared among different antibiotics using the Kruskal-Wallis test.

To evaluate and compare the possible association of PK/PD targets with therapy-related outcome, after adjusting for APACHE II and SOFA scores, the skewed logistic regression technique was used [18]. The odds ratios (ORs) and bootstrapped 95% confidence intervals (CIs) were obtained, and the model fits were assessed using Akaike information criteria and Bayesian information criteria. Based on the estimated probabilities from the above models, the area under the receiver operating characteristic curve (AUROC) with 95% CIs was estimated.

The probability of a positive clinical outcome associated with the ratio of concentration to MIC in interaction with higher and lower levels of sickness severity (APACHE II score) were evaluated using an interaction-based logistic regression setup. High and low sickness severity groups were categorized into first and third quartiles. Graphical presentation of the confidence bounds for the probability of positive clinical outcome associated with concentration to MIC ratio were developed using these groups.

RESULTS

Demographic and Clinical Data

In 68 ICUs across 10 countries, 384 patients receiving β-lactam antibiotics were identified. Twenty-three patients were excluded because of protocol violations relating to incorrect blood sample timing, leaving 361 evaluable patients. The demographic and clinical details for the patients are described in Table 2.

Table 2. Clinical and Demographic Characteristics of Included Patients

Characteristic	All Patients (n = 361)	Patients Treated for Infection (n = 248)
Male sex, %	65	65
Age, y	61 (48–73)	60 (48–74)
Weight, kg	75 (65–85)	78 (65–86)
APACHE II score	18 (13–24)	18 (14–24)
SOFA score	5 (2–9)	6 (3–9)
Serum creatinine concentration, μmol/L	77 (53–134)	76 (53–144)
Calculated creatinine clearance, mL/min	80 (42–125)	82 (44–125)
Urinary creatinine clearance, mL/min	62 (31–107)	64 (32–103)

Data are presented as median (interquartile range) unless otherwise specified.

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment.

Antibiotics were mostly used for treatment of infection (68.7%), with the remainder defined as therapy for prophylaxis of infection (31.3%). One-third of patients (32.6%) had their antibiotic course commenced in the 24 hours prior to blood sampling.

PK/PD Data

The data describing the achievement of PK/PD targets with empiric dosing are described in Table 3. The box-and-whisker plots in Figure 1 show up to 500-fold variations in the unbound concentrations of some antibiotics at both the 50% and 100% sampling times. As shown by the boxplots in Figure 2, this concentration variation also extended to variation of PK/PD indices at both time points.

Clinical Outcome Data

The clinical cure (positive clinical outcome) rate across patients receiving both treatment and prophylaxis with β-lactam antibiotics was 66.5%. The most common indications for β-lactam therapy were lung infection (41%) and intra-abdominal infections (14%). By day 30 following study enrollment, 21.9% of patients had died; 40.8% of these deaths were considered to be related to the infection. The total infection-related mortality for all patients was 8.9%.

Among those treated for infection (n = 248), 144 (58.1%) patients had a positive clinical outcome. Of the patients treated for infection, 72.9% had a bacterial pathogen isolated, of which 34.2% had a pathogen MIC available. Of the pathogens identified, 18% were *Pseudomonas aeruginosa* (median MIC, 8 mg/L [IQR, 2–16]) and 16% were *Escherichia coli* (median MIC, 4 mg/L [IQR 1–16]). The rates of positive clinical outcomes for these groups were 66% where no pathogen was isolated, 57% where 1 pathogen was isolated, and 54% for polymicrobial infections. β-Lactam monotherapy treatment was used in 38% of patients (n = 67), of whom 50% of patients achieved a positive clinical outcome (compared with the 63% in the combination therapy group).

Sixty-seven percent of patients being treated for infection received therapy by intermittent bolus dosing and 33% by prolonged infusion (either an extended infusion ≥2 hours or a continuous infusion). Of the patients who received prolonged infusion, 7% did not achieve 50% $f\,T_{>MIC}$ compared with 20% of patients receiving intermittent infusion.

Sixteen percent of patients treated for infection did not achieve 50% $f\,T_{>MIC}$, and these patients were 32% less likely to have a positive clinical outcome (OR, 0.68 [95% CI,.52–.91]; $P = .009$).

In our multivariate regression models, only APACHE II score, SOFA score, and the PK/PD indices 50% $f\,T_{>MIC}$ and 100% $f\,T_{>MIC}$ were significantly associated with the clinical outcome ($P < .05$). The median APACHE II score for patients with

Table 3. Antibiotic Data for Achievement of Pharmacokinetic/Pharmacodynamic Targets[a] in Critically Ill Patients

Dosing and PK/PD Data	Antibiotic (No. of Patients)								Total (N = 361)
	Amoxicillin (n = 71)	Ampicillin (n = 18)	Cefazolin (n = 14)	Cefepime (n = 14)	Ceftriaxone (n = 33)	Doripenem (n = 13)	Piperacillin (n = 109)	Meropenem (n = 89)	
Dosage per 24 h[b], g	6.0 (3.5–6.0)	12.0 (8.3–12.0)	3.0 (3.0–4.0)	6.0 (5.0–6.0)	2.0 (2.0–4.0)	1.75 (1.50–3.0)	12.0 (12.0–16.0)	3.0 (3.0–4.0)	
50% $f\,T_{>MIC}$ achieved	52.1%	55.6%	100.0%	78.6%	97.0%	100.0%	80.6%	95.0%	78.9%
50% $f\,T_{>4\times MIC}$ achieved	16.9%	27.8%	50.0%	50.0%	93.9%	69.2%	48.9%	68.8%	48.9%
100% $f\,T_{>MIC}$ achieved	18.3%	33.3%	78.6%	78.6%	93.9%	76.9%	67.0%	69.7%	60.4%
100% $f\,T_{>4\times MIC}$ achieved	11.3%	22.2%	14.3%	71.4%	87.9%	30.8%	30.3%	41.6%	35.0%

Abbreviation: PK/PD, pharmacokinetic/pharmacodynamic.

[a] See Table 1 for definitions of targets.

[b] Median (interquartile range).

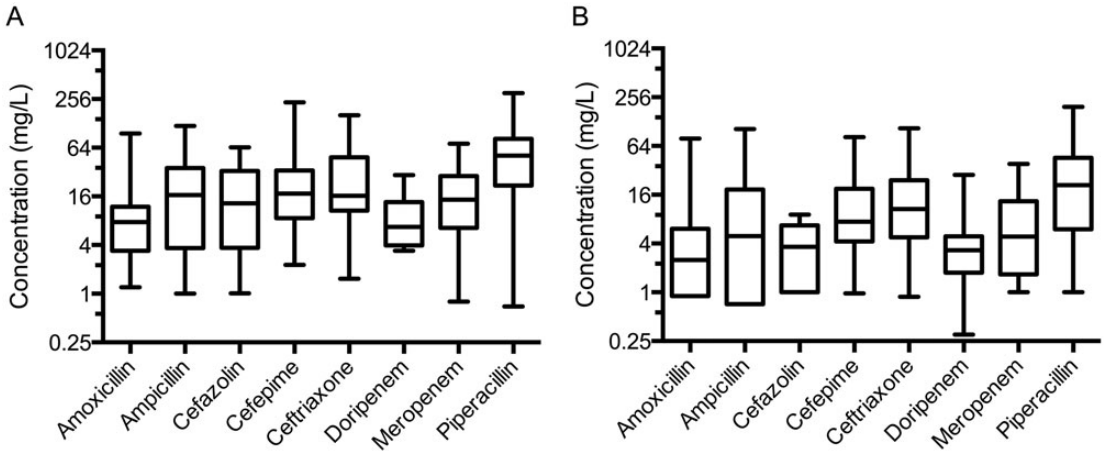

Figure 1. The boxplot of antibiotic concentrations observed at 50% (*A*) and 100% (*B*) of the dosing interval. Median, interquartile range, and range are presented. The y-axes are presented on a $\log_2$ scale.

positive and negative clinical outcomes was 18 (IQR, 13–23) and 21 (IQR, 16–27), respectively ($P < .01$). An increase in APACHE II score by 1 point was significantly associated with a 5% increased risk of negative outcome (OR, 1.05 [95% CI, 1.02–1.07]). For the 50% $f\,T_{>MIC}$ and 100% $f\,T_{>MIC}$ data, a higher PK/PD ratio was associated with higher likelihood of a positive clinical outcome (OR, 1.02 [95% CI, 1.01–1.04] and OR, 1.56 [95% CI, 1.15–2.13], respectively). The results for the model for the 220 patients who did not receive renal replacement therapy are shown in Table 4.

The predictive value of the 50% $f\,T_{>MIC}$ and 100% $f\,T_{>MIC}$ ratio for positive clinical outcome was the same (AUROC, 0.63 [95% CI, .56–.71] and 0.63 [95% CI, .56–.71]) for 50% $f\,T_{>MIC}$ and 100% $f\,T_{>MIC,}$ respectively.

The analyses of interaction effects of sickness severity status and increasing 50% $f\,T_{>MIC}$ ratios on the clinical outcome revealed that the likelihood of positive clinical outcome is significantly higher with increasing level of ratio of antibiotic concentration to MIC for those with lower APACHE II score, compared to those with higher APACHE II score (Figure 3*A* and 3*B*).

We also examined the effect of 50% $f\,T_{>MIC}$ on positive clinical outcome for different types of infection. For bloodstream infections (n = 24), a significant association was clearly present with increasing antibiotic concentrations at 50% of the dosing

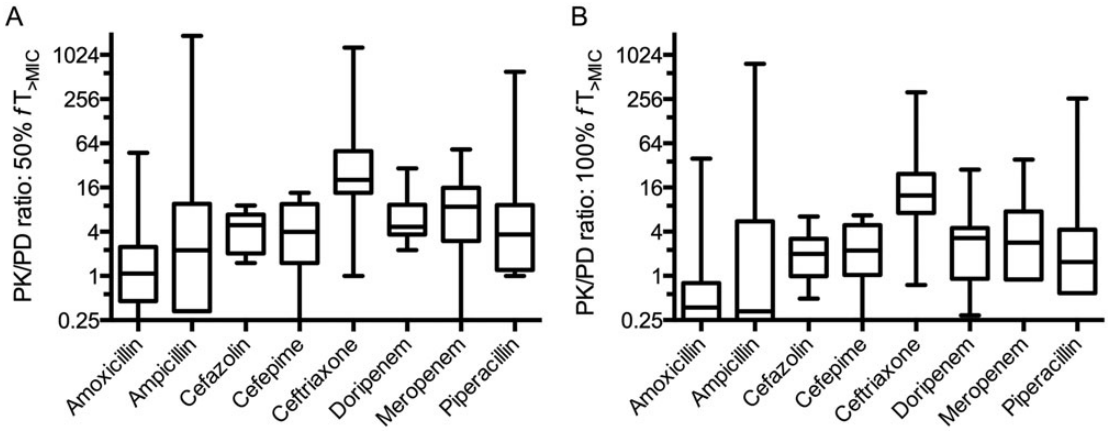

Figure 2. The pharmacokinetic/pharmacodynamic (PK/PD) ratios observed at 50% (*A*) and 100% (*B*) of the dosing interval. A ratio of 1 is considered to be a minimum PK/PD target of therapy at 50% of the dosing interval. Note that the PK/PD ratio is defined as the ratio between the measured antibiotic concentration in plasma at 50% or 100% of the dosing interval and the patient's minimum inhibitory concentration (MIC) or surrogate when MIC or pathogen is unknown. Abbreviation: $f\,T_{>MIC}$, time the free (unbound) antibiotic concentration was maintained above the minimum inhibitory concentration.

Table 4. Multivariate Regression Results of Clinical Outcome for Patients Who Did Not Receive Renal Replacement Therapy

	50% $fT_{>MIC}$			100% $fT_{>MIC}$		
Model Parameters	OR	95% CI	*P* Value	OR	95% CI	*P* Value
APACHE II score	0.94	.92–.96	<.001	0.94	.92–.96	.97
SOFA score	0.97	.94–1.00	.053	0.97	.94–1.01	.13
50% $fT_{>MIC}$	1.03	1.01–1.04	.001	. . .		
100% $fT_{>MIC}$	. . .			1.02	1.01–1.05	.040
AIC	1758.60					
BIC	1785.07					

Data are presented as estimates of odds ratios (95% CI) and *P* values. Abbreviations: AIC, Akaike information criteria; APACHE, Acute Physiology and Chronic Health Evaluation II; BIC, Bayesian information criteria; CI, confidence interval; $fT_{>MIC}$, time the free (unbound) antibiotic concentration was maintained above the minimum inhibitory concentration; OR, odds ratio; SOFA, Sequential Organ Failure Assessment.

interval, resulting in a greater probability of positive clinical outcome (OR, 1.13 [95% CI, 1.07–1.19]). However, neither lung infection (n = 104; OR, 0.99 [95% CI,.99–1.00]) nor intra-abdominal infection (n = 35; OR, 1.01 [95% CI,.96–1.06]) showed any significant associations.

The mean and median levels of MIC for all suspected bacteria in all patients were 8 mg/L and 2 mg/L, respectively. The patients with a pathogen with a MIC ≤2 mg/L were 2.3 times more likely to achieve a positive clinical outcome (OR, 2.27 [95% CI, 1.79–2.87]).

DISCUSSION

This multinational point prevalence study is the first to examine unbound plasma concentrations of β-lactam antibiotics and patient outcome across a large number of ICUs. These data show that mid-dose and trough β-lactam concentrations vary widely, and as such, achievement of PK/PD targets is highly inconsistent. Of great concern, one-fifth of patients do not even achieve a minimum conservative PK/PD target, 50% $fT_{>MIC}$. This study has also generated interesting hypotheses related to much higher target β-lactam pharmacokinetic exposures than would have been previously considered for clinical outcome of infection. The dictum of "one dose fits all" is shown here to be problematic.

Our finding of large variations in plasma concentrations of β-lactam antibiotics in ICUs is in keeping with other studies. Recent reviews have noted the enormous pharmacokinetic variability of β-lactam antibiotics in critically ill patients [6, 7]; however, all the studies commented on in these reviews were derived from single centers or from only very few related centers.

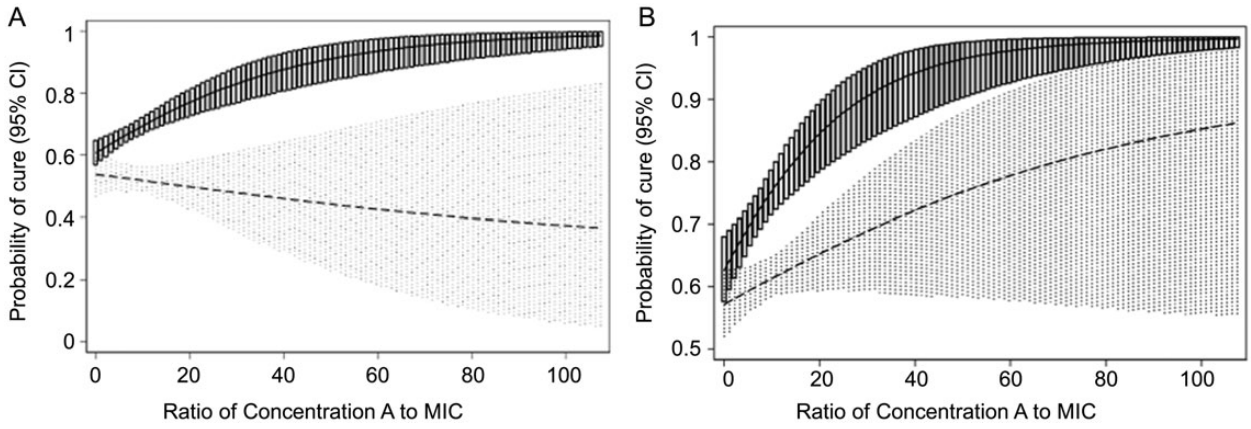

Figure 3. *A*, The effect of an increasing pharmacokinetic/pharmacodynamic (PK/PD) ratio at 50% of the dosing interval (ratio A) in interaction with Acute Physiology and Chronic Health Evaluation II (APACHE II) score on the probability of positive clinical outcome (n = 248; y-axis). *B*, The effect of PK/PD ratio at 50% of the dosing interval (ratio A) in interaction with APACHE II score on the probability of positive clinical outcome for patients not receiving renal replacement therapy (n = 220; y-axis). The estimated probabilities of positive clinical outcome along with its 95% confidence interval are presented for the less critically ill patient group (APACHE II score within lowest quartile of 0–14 points; solid black lines) as well as the more critically ill patient group (APACHE II score within the third quartile of 18–24 points; dashed line). Abbreviations: CI, confidence interval; MIC, minimum inhibitory concentration.

These studies are valuable because they demonstrate how antibiotic concentrations in different types of patients will differ from non–critically ill patients. Such data are essential for articulating how antibiotic dosing regimens that meet the specific needs of these patients could be developed given that present regimens are not tested in these most severely ill patients by pharmaceutical companies.

Antibiotics discovered and evaluated in vitro are tested in animals initially for toxicity, and subsequently for efficacy. The antibiotic dose and frequency are based on these in vitro or animal in vivo PK/PD studies. These dosing regimens are then tested on healthy human volunteers for tolerability, with clinical efficacy studies undertaken in non–critically ill patients. After the launch of the drug onto the general market, the same dosing regimen is used in critically ill patients; however, this is likely to lead to suboptimal outcomes in the ICU. Critically ill patients have altered volumes of distribution for antibiotics [19, 20], and, unlike other patient groups, need larger initial doses to rapidly achieve therapeutic concentrations [21]. These patients may have augmented renal clearances needing either higher doses or more frequent dosing to overcome increased drug elimination [22, 23]. Critically ill patients often have low plasma albumin concentrations [24] that alters the protein binding of drugs and has significant effects on pharmacokinetics [25, 26].

Given such potential for variability, it is not surprising that we found that one-fifth of patients did not achieve the most conservative PK/PD target and <50% of patients achieved what we a priori defined as a preferred PK/PD target (Table 3). Furthermore, the variability of unbound concentrations across all antibiotics (Figure 1) as well as PK/PD ratios (Figure 2) were similarly large.

The consequences of insufficient antibiotic exposure may be severe, with clear relationships being demonstrated between antibiotic underdosing and the development of antibiotic resistance [27]. This link was initially shown with inappropriately low quinolone exposures [28], but more recently with other classes of antibiotics including β-lactams [29, 30]. ICUs are known to harbor multidrug-resistant pathogens and, although there are many reasons for this, optimized dosing that minimizes the evolution of such pathogens should be considered a method to improve patient and health system outcomes.

The secondary objective of this study was to compare antibiotic PK/PD with observed clinical outcomes. An interesting finding in our study is the observed significant interaction effect of varying sickness severity while evaluating the dose-response relationship. The patterns of probability of positive clinical outcome associated with increasing level of PK/PD ratio were markedly different for higher and lower levels of disease severity levels (Figure 3). This novel analysis approach delineates the effect of antibiotic exposure more accurately. We found that the magnitude of the β-lactam exposures necessary to achieve a positive clinical outcome is particularly noteworthy and generates interesting research questions for future study.

The results of the DALI study support the conclusions of previous small studies that better outcomes for critically ill patients can be expected with higher drug exposures, at least for less severely ill patients [12, 13]. These data now support the conduct of an interventional study comparing critically ill patient outcomes with different PK/PD targets to definitively determine what antibiotic exposures should be targeted in these patients.

This study has notable limitations. While it is a prospectively designed point prevalence study, it is merely a snapshot picture of β-lactam antibiotic concentrations in critically ill patients on a single day [14]. Although we collected data on concomitant antibiotics, we did not assess the PK/PD of those antibiotics, nor did we assess duration of therapy of combination or monotherapy. Furthermore, pathogens were only grown in 73% of patients, and the actual MIC was only available in 34% of these patients, meaning that assumptions were necessary for the remaining patients. Such assumptions were of a worst-case scenario, which we believe is highly acceptable as this is the context governing empiric dose selection. If the infections were mediated by more susceptible bacteria than were assumed, the PK/PD ratios would actually have been higher than those we described here. Finally, we have not specifically looked at drug concentrations at the site of infection, because of the technical challenges in performing such a large-scale evaluation. However, our data interestingly show that for bloodstream infections, where antibiotic concentrations were measured, a strong PK/PD relationship was present.

CONCLUSIONS

The implications of this large study performed across 68 ICUs are profound. These data show that many patients fall below PK/PD targets (less than the most conservative PK/PD target in 20% of patients, and less than our suggested target in 50%). The results suggest that ICU clinicians should refine dosing strategies for critically ill patients to optimize β-lactam antibiotic outcomes. With the significant pharmacokinetic variability observed, a more personalized approach to antibiotic dosing would need to be adopted to ensure that target drug exposures are assured.

Notes

Author contributions. J. A. R. and J. L. designed the study and wrote the initial protocol. S. K. P. and J. A. R. performed the modeling and data analyses. All authors helped conduct the trial and provided advice and input into the design and interpretation of the data and the drafting of the manuscript.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA **2009**; 302: 2323–9.
2. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med **2006**; 34: 1589–96.
3. Harbarth S, Garbino J, Pugin J, Romand JA, Lew D, Pittet D. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. Am J Med **2003**; 115: 529–35.
4. MacArthur RD, Miller M, Albertson T, et al. Adequacy of early empiric antibiotic treatment and survival in severe sepsis: experience from the MONARCS trial. Clin Infect Dis **2004**; 38: 284–8.
5. Udy AA, Roberts JA, Dewaele J, Paterson DL, Lipman J. What's behind the failure of emerging antibiotics in the critically ill? Understanding the impact of altered pharmacokinetics and augmented renal clearance. Int J Antimicrob Agents **2012**; 39: 455–7.
6. Goncalves-Pereira J, Povoa P. Antibiotics in critically ill patients: a systematic review of the pharmacokinetics of beta-lactams. Crit Care **2011**; 15: R206.
7. Sime F, Roberts MS, Peake SL, Lipman J, Roberts JA. Does beta-lactam pharmacokinetic variability in critically ill patients justify therapeutic drug monitoring? A systematic review. Ann Intensive Care **2012**; 2: 35.
8. Lipman J, Udy AA, Roberts JA. Do we understand the impact of altered physiology, consequent interventions, and resultant clinical scenarios in ICU? The antibiotic story. Anaesth Intens Care **2011**; 39: 999–1000.
9. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis **1998**; 26: 1–10; quiz 1–2.
10. Mouton JW, den Hollander JG. Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an in vitro pharmacokinetic model. Antimicrob Agents Chemother **1994**; 38: 931–6.
11. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug.' Nat Rev Microbiol **2004**; 2: 289–300.
12. Li C, Du X, Kuti JL, Nicolau DP. Clinical pharmacodynamics of meropenem in patients with lower respiratory tract infections. Antimicrob Agents Chemother **2007**; 51: 1725–30.
13. McKinnon PS, Paladino JA, Schentag JJ. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration ($T_{>MIC}$) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents **2008**; 31: 345–51.
14. Roberts JA, De Waele JJ, Dimopoulos G, et al. DALI: Defining Antibiotic Levels in Intensive care unit patients: a multi-centre point of prevalence study to determine whether contemporary antibiotic dosing for critically ill patients is therapeutic. BMC Infect Dis **2012**; 12: 152.
15. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med **1985**; 13: 818–29.
16. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med **1998**; 26: 1793–800.
17. Briscoe SE, McWhinney BC, Lipman J, Roberts JA, Ungerer JP. A method for determining the free (unbound) concentration of ten beta-lactam antibiotics in human plasma using high performance liquid chromatography with ultraviolet detection. J Chromatogr **2012**; 907: 178–84.
18. Nagler J. Scobit: an alternative estimator to logit and probit. Am J Pol Sci **1994**; 38: 230–55.
19. Marik PE. Aminoglycoside volume of distribution and illness severity in critically ill septic patients. Anaesth Intensive Care **1993**; 21: 172–3.
20. Gous AG, Dance MD, Lipman J, Luyt DK, Mathivha R, Scribante J. Changes in vancomycin pharmacokinetics in critically ill infants. Anaesth Intensive Care **1995**; 23: 678–82.
21. Roberts JA, Taccone FS, Udy AA, Vincent JL, Jacobs F, Lipman J. Vancomycin dosing in critically ill patients: robust methods for improved continuous-infusion regimens. Antimicrob Agents Chemother **2011**; 55: 2704–9.
22. Udy AA, Roberts JA, Boots RJ, Paterson DL, Lipman J. Augmented renal clearance: implications for antibacterial dosing in the critically ill. Clin Pharmacokinet **2010**; 49: 1–16.
23. Udy AA, Varghese JM, Altukroni M, et al. Sub-therapeutic initial beta-lactam concentrations in select critically ill patients: association between augmented renal clearance and low trough drug concentrations. Chest **2012**; 142: 30–9.
24. Finfer S, Bellomo R, McEvoy S, Lo SK, Myburgh J, Neal B, et al. Effect of baseline serum albumin concentration on outcome of resuscitation with albumin or saline in patients in intensive care units: analysis of data from the saline versus albumin fluid evaluation (SAFE) study. BMJ **2006**; 333: 1044.
25. Ulldemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimising antibiotic dosing in critically ill patients. Clin Pharmacokinet **2011**; 50: 1–12.
26. Roberts JA, Pea F, Lipman J. The clinical relevance of plasma protein binding changes. Clin Pharmacokinet **2013**; 52: 1–8.
27. Roberts JA, Kruger P, Paterson DL, Lipman J. Antibiotic resistance—what's dosing got to do with it? Crit Care Med **2008**; 36: 2433–40.
28. Stamey TA, Bragonje J. Resistance to nalidixic acid. A misconception due to underdosage. JAMA **1976**; 236: 1857–60.
29. Fantin B, Farinotti R, Thabaut A, Carbon C. Conditions for the emergence of resistance to cefpirome and ceftazidime in experimental endocarditis due to *Pseudomonas aeruginosa*. J Antimicrob Chemother **1994**; 33: 563–9.
30. Gugel J, Dos Santos Pereira A, Pignatari AC, Gales AC. Beta-lactam MICs correlate poorly with mutant prevention concentrations for clinical isolates of *Acinetobacter* spp. and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother **2006**; 50: 2276–7.

Appendix. DALI (Defining Antibiotic Levels in Intensive Care) Study Group

Author Name	Affiliation
Jason A. Roberts	Burns Trauma and Critical Care Research Centre, The University of Queensland, Brisbane, Australia Royal Brisbane and Women's Hospital, Brisbane, Australia
Jeffrey Lipman	Burns Trauma and Critical Care Research Centre, The University of Queensland, Brisbane, Australia Royal Brisbane and Women's Hospital, Brisbane, Australia
Therese Starr	Royal Brisbane and Women's Hospital, Brisbane, Australia
Steven C. Wallis	Burns Trauma and Critical Care Research Centre, The University of Queensland, Brisbane, Australia
Sanjoy K. Paul	Queensland Clinical Trials & Biostatistics Centre, School of Population Health, The University of Queensland, Brisbane, Australia
Antonio Margarit Ribas	Hospital Nostra Senyora de Meritxell, Escaldes-Engordany, Andorra
Jan J. De Waele	Ghent University Hospital, Ghent, Belgium
Luc De Crop	Ghent University Hospital, Ghent, Belgium
Herbert Spapen	Universitair Ziekenhuis Brussels, Brussels, Belgium
Joost Wauters	Universitair Ziekenhuis Gasthuisberg, Leuven, Brussels
Thierry Dugernier	Clinique Saint Pierre, Ottignies, Belgium
Philippe Jorens	Universitair Ziekenhuis Antwerpen, Edegem, Belgium
Ilse Dapper	Algemeen Ziekenhuis Monica, Deurne, Belgium
Daniel De Backer	Erasme University Hospital, Brussels, Belgium
Fabio S. Taccone	Erasme University Hospital, Brussels, Belgium
Jordi Rello	Vall d'Hebron Institut of Research. Universitat Autonoma de Barcelona, Spain Centro de Investigación Biomedica En Red- Enfermedades Respiratorias (CibeRes)
Laura Ruano	Vall d'Hebron Institut of Research; Universitat Autonoma de Barcelona, Spain Centro de Investigación Biomedica En Red- Enfermedades Respiratorias (CibeRes)
Elsa Afonso	Hospital Universitari Vall d'Hebron; Vall d'Hebron Institut of Research. Universitat Autonoma de Barcelona, Spain Centro de Investigación Biomedica En Red- Enfermedades Respiratorias (CibeRes)
Francisco Alvarez-Lerma	Hospital Del Mar, Parc Salut Mar. Barcelona, Spain
Maria Pilar Gracia-Arnillas	Hospital Del Mar, Parc Salut Mar. Barcelona, Spain
Francisco Fernández	Centro Médico Delfos, Barcelona
Neus Feijoo	Hospital General de L'Hospitalet, Barcelona, Spain
Neus Bardolet	Hospital General de L'Hospitalet, Barcelona, Spain
Assumpta Rovira	Hospital General de L'Hospitalet, Barcelona, Spain
Pau Garro	Hospital General de Granollers, Barcelona, Spain
Diana Colon	Hospital General de Granollers, Barcelona, Spain
Carlos Castillo	Hospital Txagorritxu, Vitoria, Spain
Juan Fernado	Hospital Txagorritxu, Vitoria, Spain
Maria Jesus Lopez	Hospital Universitario de Burgos. Burgos, Spain
Jose Luis Fernandez	Hospital Universitario de Burgos. Burgos, Spain
Ana Maria Arribas	Hospital Universitario de Burgos. Burgos, Spain
Jose Luis Teja	Hospital Universitario Marques de Valdecilla, Santander, Spain
Elsa Ots	Hospital Universitario Marques de Valdecilla, Santander, Spain
Juan Carlos Montejo	Hospital Universitario 12 de Octubre, Madrid, Spain
Mercedes Catalan	Hospital Universitario 12 de Octubre, Madrid, Spain
Isidro Prieto	Hospital Ramon y Cajal, Madrid, Spain
Gloria Gonzalo	Hospital Ramon y Cajal, Madrid, Spain
Beatriz Galvan	Hospital Universitario La Paz, Madrid, Spain
Miguel Angel Blasco	Hospital Universitario Severo Ochoa, Madrid, Spain
Estibaliz Meyer	Hospital Universitario Severo Ochoa, Madrid, Spain
Frutos Del Nogal	Hospital Universitario Severo Ochoa, Madrid, Spain
Loreto Vidaur	Hospital Universitario de Donostia, Donostia, Spain
Rosa Sebastian	Hospital Universitario de Donostia, Donostia, Spain
Pila Marco Garde	Hospital Universitario de Donostia, Donostia, Spain

Appendix continued.

Author Name	Affiliation
Maria del Mar Martin Velasco	Hospital Universitario Nuestra Señora de Candelaria, Spain
Rafael Zaragoza Crespo	Hospital Universitario Dr. Peset, Spain
Mariano Esperatti	Institut Clínic del Tòrax, Hospital Clinic, IDIBAPS, Barcelona, Spain; Centro de Investigación Biomedica En Red-Enfermedades Respiratorias (CibeRes)
Antoni Torres	Institut Clínic del Tòrax, Hospital Clinic, IDIBAPS, Barcelona, Spain; Centro de Investigación Biomedica En Red- Enfermedades Respiratorias (CibeRes)
Philippe Montravers	Centre Hospitalier Universitaire Bichat-Claude Bernard, AP-HP, Université Paris VII, Paris, France
Olivier Baldesi	Centre Hospitalier Pays D Aix, Aix en Provence, France and AzuRea Group
Herve Dupont	Centre Hospitalier Universitaire d'Amiens, Amiens, France
Yazine Mahjoub	Centre Hospitalier-Universitaire d'Amiens, Amiens, France and AzuRea Group
Sigismond Lasocki	Centre Hospitalier-Universitaire d'Angers, Angers, France
Jean Michel Constantin	Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont-Ferrand, France and AzuRea Group
Jean François Payen	Centre Hospitalier-Universitaire Grenoble, Grenoble France and AzuRea Group
Claude Martin	Hopital Nord, Marseille, France; AzuRea Group, France
Jacques Albanese	Hopital Nord, Marseille, France and AzuRea Group
Yannick Malledant	Hôpital Pontchaillou, Rennes, France
Julien Pottecher	University Hospital, Strasbourg, France and AzuRea Group
Jean-Yves Lefrant	Centre Hospitalier-Universitaire Nimes, Nimes France and AzuRea Group
Samir Jaber	Hospitalier-Universitaire Montpellier, Montpellier, France and AzuRea Group
Olivier Joannes-Boyau	Centre Hospitalier-Universitaire Bordeaux, Bordeaux, France and AzuRea Group
Christophe Orban	Centre Hospitalier-Universitaire Nice, Nice, France and AzuRea Group
Marlies Ostermann	St Thomas' Hospital, London, United Kingdom
Cathrine McKenzie	St Thomas' Hospital, London, United Kingdom
William Berry	St Thomas' Hospital, London, United Kingdom
John Smith	St Thomas' Hospital, London, United Kingdom
Katie Lei	St Thomas' Hospital, London, United Kingdom
Francesca Rubulotta	Charing Cross Hospital, Imperial Healthcare NHS Trust, London, United Kingdom
Anthony Gordon	Charing Cross Hospital, Imperial Healthcare NHS Trust, London, United Kingdom
Stephen Brett	Hammersmith Hospital, Imperial Healthcare NHS Trust, London, United Kingdom
Martin Stotz	St Mary's Hospital, Imperial Healthcare NHS Trust, London, United Kingdom
Maie Templeton	St Mary's Hospital, Imperial Healthcare NHS Trust, London, United Kingdom
Andrew Rhodes	St George's Hospital, St George's Healthcare NHS Trust, London, United Kingdom
Claudia Ebm	St George's Hospital, St George's Healthcare NHS Trust, London, United Kingdom
Carl Moran	St George's Hospital, St George's Healthcare NHS Trust, London, United Kingdom
Kirsi-Maija Kaukonen	Helsinki University Central Hospital, Helsinki, Finland
Ville Pettilä	Helsinki University Central Hospital, Helsinki, Finland
George Dimopoulos	Attikon University Hospital, Athens, Greece
Despoina Koulenti	Attikon University Hospital, Athens, Greece
Aglaia Xristodoulou	Attikon University Hospital, Athens, Greece
Vassiliki Theodorou	University Hospital of Alexandroupolis, Alexandroupolis, Greece
Georgios Kouliatsis	University Hospital of Alexandroupolis, Alexandroupolis, Greece
Eleni Sertaridou	University Hospital of Alexandroupolis, Alexandroupolis, Greece
Georgios Anthopoulos	251 Air Force General Hospital of Athens, Athens, Greece
George Choutas	251 Air Force General Hospital of Athens, Athens, Greece
Thanos Rantis	251 Air Force General Hospital of Athens, Athens, Greece
Stylianos Karatzas	General Hospital of Athens 'Hippokrateion,' Athens, Greece
Margarita Balla	General Hospital of Athens 'Hippokrateion,' Athens, Greece
Metaxia Papanikolaou	General Hospital of Athens 'Hippokrateion,' Athens, Greece
Pavlos Myrianthefs	'Aghioi Anargyroi' Hospita, Athens, Greece
Alexandra Gavala	'Aghioi Anargyroi' Hospita, Athens, Greece
Georgios Fildisis	'Aghioi Anargyroi' Hospita, Athens, Greece

Appendix continued.

Author Name	Affiliation
Antonia Koutsoukou	Sotiria General Hospital, Athens, Greece
Magdalini Kyriakopoulou	Sotiria General Hospital, Athens, Greece
Kalomoira Petrochilou	Sotiria General Hospital, Athens, Greece
Maria Kompoti	'Thriassio' General Hospital of Eleusi, Athens, Greece
Martha Michalia	'Thriassio' General Hospital of Eleusi, Athens, Greece
Fillis-Maria Clouva-Molyvdas	'Thriassio' General Hospital of Eleusi, Athens, Greece
Georgios Gkiokas	Aretaieion University Hospital, Athens, Greece
Fotios Nikolakopoulos	Aretaieion University Hospital, Athens, Greece
Vasiliki Psychogiou	Aretaieion University Hospital, Athens, Greece
Polychronis Malliotakis	University Hospital Herakleion, Crete, Greece
Evangelia Akoumianaki	University Hospital Herakleion, Crete, Greece
Emmanouil Lilitsis	University Hospital Herakleion, Crete, Greece
Vassilios Koulouras	University Hospital of Ioannina, Ioannina, Greece
George Nakos	University Hospital of Ioannina, Ioannina, Greece
Mihalis Kalogirou	University Hospital of Ioannina, Ioannina, Greece
Apostolos Komnos	General Hospital of Larisa, Larisa, Greece
Tilemachos Zafeiridis	General Hospital of Larisa, Larisa, Greece
Christos Chaintoutis	General Hospital of Larisa, Larisa, Greece
Kostoula Arvaniti	General Hospital of Thessaloniki 'G. Papageorgiou,' Thessaloniki, Greece
Dimitrios Matamis	General Hospital of Thessaloniki 'G. Papageorgiou,' Thessaloniki, Greece
Christos Chaintoutis	General Hospital of Thessaloniki 'G. Papageorgiou,' Thessaloniki, Greece
Christina Kydona	General Hospital of Thessaloniki 'Hippokrateion,' Thessaloniki, Greece
Nikoleta Gritsi-Gerogianni	General Hospital of Thessaloniki 'Hippokrateion,' Thessaloniki, Greece
Tatiana Giasnetsova	General Hospital of Thessaloniki 'Hippokrateion,' Thessaloniki, Greece
Maria Giannakou	Ahepa University Hospital, Thessaloniki, Greece
Ioanna Soultati	Ahepa University Hospital, Thessaloniki, Greece
Ilias Chytas	Ahepa University Hospital, Thessaloniki, Greece
Eleni Antoniadou	General Hospital 'G. Gennimatas,' Thessaloniki, Greece
Elli Antipa	General Hospital 'G. Gennimatas,' Thessaloniki, Greece
Dimitrios Lathyris	General Hospital 'G. Gennimatas,' Thessaloniki, Greece
Triantafyllia Koukoubani	General Hospital of Trikala, Trikala, Greece
Theoniki Paraforou	General Hospital of Trikala, Trikala, Greece
Kyriaki Spiropoulou	General Hospital of Trikala, Trikala, Greece
Vasileios Bekos	Naval Hospital of Athens, Athens, Greece
Anna Spring	Naval Hospital of Athens, Athens, Greece
Theodora Kalatzi	Naval Hospital of Athens, Athens, Greece
Hara Nikolaou	'Aghia Olga-Konstantopouleion' General Hospital, Athens, Greece
Maria Laskou	'Aghia Olga-Konstantopouleion' General Hospital, Athens, Greece
Ioannis Strouvalis	'Aghia Olga-Konstantopouleion' General Hospital, Athens, Greece
Stavros Aloizos	General Hospital of Athens 'NIMITS,' Athens, Greece
Spyridon Kapogiannis	General Hospital of Athens 'NIMITS,' Athens, Greece
Ourania Soldatou	General Hospital of Athens 'NIMITS,' Athens, Greece
Matteo Bassetti	Azienda Ospedaliera Universitaria Santa Maria della Misericordia, Udine, Italy
Chiara Adembri	Azienda Ospedaliero Universitaria Careggi, Florence, Italy
Gianluca Villa	Azienda Ospedaliero Universitaria Careggi, Florence, Italy
Antonio Giarratano	Università degli Studi di Palermo, Palermo, Italy
Santi Maurizio Raineri	Università degli Studi di Palermo, Palermo, Italy
Andrea Cortegiani	Università degli Studi di Palermo, Palermo, Italy
Francesca Montalto	Università degli Studi di Palermo, Palermo, Italy
Maria Teresa Strano	Università degli Studi di Palermo, Palermo, Italy

Appendix continued.

Author Name	Affiliation
V. Marco Ranieri	San Giovanni-Battista Molinette, Turin, Italy
Claudio Sandroni	Catholic University School of Medicine, Rome, Italy
Gennaro De Pascale	Catholic University School of Medicine, Rome, Italy
Alexandre Molin	University of Genoa, Genoa, Italy
Paolo Pelosi	University of Genoa, Genoa, Italy
Luca Montagnani	Universitaria San Martino, Genova, Italy
Rosario Urbino	Universitaria S.Giovanni Battista della Città di Torino, Torino, Italy
Ilaria Mastromauro	Universitaria S.Giovanni Battista della Città di Torino, Torino, Italy
Francesco G. De Rosa	Universitaria S.Giovanni Battista della Città di Torino, Torino, Italy
V. Marco Ranieri	Universitaria S.Giovanni Battista della Città di Torino, Torino, Italy
Teresa Cardoso	Hospital de Santo António, Porto, Portugal
Susana Afonso	Hospital de St António dos Capuchos, Lisbon, Portgual
João Gonçalves-Pereira	Hospital de São Francisco Xavier, Lisbon, Portugal
João Pedro Baptista	Hospital de Universidade de Coimbra, Coimbra, Portugal
Murat Akova	Hacettepe University School of Medicine, Ankara, Turkey
Arife Özveren	Hacettepe University School of Medicine, Ankara, Turkey