A Rapid Qualitative Assay to Detect Circulating Endotoxin Can Predict the Development of Multiorgan Dysfunction*

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Objective: **To determine whether a rapid qualitative assay for the detection of circulating endotoxin (SimpliRED Endotoxin Test [SRE]; AGEN, Inc; Brisbane, Australia) can predict the occurrence of multiorgan dysfunction and hospital mortality. To compare the SRE to the limulus amebocyte lysate (LAL) assay as a predictor of clinical outcomes.**

Design: **Prospective, blinded, single-center study.**

Setting: **Medical ICU of Barnes-Jewish Hospital, St. Louis, a university-affiliated teaching hospital.**

Patients: **Included in the study were 265 adult patients requiring medical ICU admission.**

Interventions: **Daily collection of blood samples.**

Measurements and results: **Daily detection for the presence of endotoxin in blood during intensive care and assessment for the development of multiorgan dysfunction (*ie*, an organ system failure index >2) or death. On ICU day 1, 55 (20.8%) patients had circulating endotoxin detected by the SRE. On ICU day 2, 29 of the 143 (20.3%) patients remaining in the ICU had a positive SRE. The development of multiorgan dysfunction was significantly greater among SRE-positive patients (44.8%) compared to SRE-negative patients (21.9%) on ICU day 2 (p=0.013). Multiple logistic regression analysis identified a positive SRE on ICU day 2 (adjusted odds ratio, 4.1; 95% confidence interval, 2.5 to 6.8; p=0.006) as being independently associated with the development of multiorgan dysfunction. A positive SRE test was not predictive of hospital mortality. Direct quantitative measurement of the concentration of circulating endotoxin using the LAL assay was not associated with either the development of multiorgan dysfunction or hospital mortality (p>0.1).**

Conclusions: **Our preliminary data suggest that a bedside assay to qualitatively detect circulating endotoxin is predictive of the development of multiorgan dysfunction among patients admitted to a medical ICU. The rapid detection of circulating endotoxin could be useful for stratifying patients into various risk categories for the development of multiorgan dysfunction.**

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Key words: endotoxin; Gram-negative bacteria; hospital mortality; intensive care; multiorgan dysfunction; outcomes; sepsis

Abbreviations: APACHE=acute physiology and chronic health evaluation; CI=confidence interval; EU=endotoxin unit; FIO_2=fraction of inspired oxygen; LAL=limulus amebocyte lysate; OSFI=organ system failure index; ROC=receiver operating characteristic; SIRS=systemic inflammatory response syndrome; SRE=SimpliRED Endotoxin Test

The development of multiorgan dysfunction is the leading cause of morbidity and mortality among patients admitted to the ICU setting.[1-3] Systemic infection or sepsis is thought to be the most common predisposition or risk factor for the development of multiorgan dysfunction.[1,4,5] Several studies have suggested that circulating endotoxin, originating from Gram-negative bacteria, is primarily responsible for the systemic inflammatory response resulting in the development of multiorgan dysfunction.[6,7] Current efforts to prevent or reverse the consequences of sepsis due to Gram-negative bacteria, including the subsequent development of multiorgan dysfunction, have focused primarily on various antiendotoxins and anticytokines to blunt the host's inflammatory response.[8,9]

The detection of endotoxin in blood has been

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proposed as a means of identifying patients who could potentially benefit from antiendotoxin and anticytokine therapies.[10] Additionally, the demonstration of endotoxemia has been suggested to be an important prognostic variable for patients developing multiorgan dysfunction.[11,12] However, problems with the current methods of detecting and quantifying endotoxin have precluded its routine use in the ICU setting. These problems include the need for a skilled laboratory to perform these tests, variability among the various assays used to quantify the concentration of endotoxin, and the inability of quantitative measurements of endotoxin to consistently predict patient outcomes, including the development of multiorgan dysfunction.[13] To address some of these problems, we performed an investigation of a recently developed monoclonal antibody RBC agglutination assay for the qualitative detection of endotoxin (SimpliRED Endotoxin Test [SRE]; AGEN, Inc; Brisbane, Australia). Our main objective was to determine whether the SRE assay is helpful in predicting the development of either multiorgan dysfunction or hospital mortality. We also wanted to compare the predictive accuracy of the SRE with a quantitative measurement of endotoxin using the limulus amebocyte lysate (LAL) assay.

Materials and Methods

Study Location and Population

The study was conducted within the medical ICU (19 beds) of Barnes-Jewish Hospital, St. Louis, a 1,200-bed private teaching hospital. All patients admitted to the medical ICU during a 3-month period (April 1995 through June 1995) were evaluated prospectively. This study was approved by the Human Studies Committee of Washington University School of Medicine which waived the need for informed consent.

Data Collection

A clinical research nurse made daily rounds in the medical ICU during weekdays recording relevant data from patient medical records, bedside computerized nursing stations (EMTEK; EMTEK Health Care Systems Inc; Tempe, Ariz), and the hospital's mainframe computer for reports of microbiological studies (sputum Gram's stains and sputum, blood, pleural fluid, lower respiratory tract, wound, urine, and IV catheter-associated cultures). Study variables recorded at presentation to the ICU included the following: age, sex, race, APACHE II score (from acute physiology and chronic health evaluation II),[14] and the ratio of the partial pressure of arterial oxygen to the inspired concentration of oxygen (PaO_2/FIO_2). The development of the systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, or septic shock[4] and the occurrence of bacteriologically documented infections were recorded during the ICU stay. The clinical outcomes evaluated included hospital mortality, ICU length of stay, hospital length of stay, and the development of individual organ system derangements.

Definitions

All definitions were selected prospectively as part of the original study design. The definitions used for SIRS, sepsis, severe sepsis, and septic shock were those proposed by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference.[4] SIRS was defined as two or more of the following: temperature >38°C or <36°C; heart rate >90 beats/min; respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg; and WBC count $>12.0\times10^9$/L, $<4.0\times10^9$/L, or the presence of >0.10 immature forms (*ie*, bands). Sepsis included SIRS plus a clinically identified site of infection (*eg*, pneumonia, urinary tract). Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion abnormalities, or hypotension (reduction of systolic pressure to <90 mm Hg or a reduction of ≥40 mm Hg from baseline in the absence of other causes for hypotension). Hypoperfusion abnormalities included, but were not limited to, lactic acidosis, oliguria, and the acute alteration in mental status. Septic shock was defined as persistent hypotension associated with hypoperfusion abnormalities despite the administration of IV fluids (*ie*, ≥500 mL fluid bolus). Patients requiring >5 μg/kg/min of dopamine or any dose of norepinephrine to maintain a systolic BP ≥90 mm Hg were also classified as having septic shock.

Organ system derangements were defined according to the organ system failure index (OSFI).[15] One point was given for dysfunction of each organ system using the following definitions: renal, a twofold increase in the baseline creatinine level or an absolute increase in the creatinine level by 176.8 μmol/L (2.0 mg/dL); hepatic, a rise in the total bilirubin level to >34.2 μmol/L (2.0 mg/dL); pulmonary, requiring mechanical ventilation for a diagnosis of pneumonia, COPD, asthma, pulmonary edema (cardiogenic or noncardiogenic), or PaO_2 <60 mm Hg while receiving an FIO_2 ≥0.50, or the use of 10 cm H_2O or more of positive end-expiratory pressure; bone marrow, the presence of disseminated intravascular coagulation, WBC count of $<1.0\times10^9$/L, or a platelet count of $<75\times10^9$/L; neurologic, new focal deficit (*eg*, hemiparesis following cerebral infarction) or new generalized process (*eg*, seizures or coma); GI, GI hemorrhage requiring transfusion, new ileus, or diarrhea lasting >24 h and unrelated to prior bowel surgery; and cardiac, acute myocardial infarction, cardiac arrest, or the new onset of congestive heart failure. We used an OSFI of >2 to define multiorgan dysfunction.[3,16] The presence of infection, including the site and etiologic agent of infection, was determined by the physicians caring for the patients as documented in the medical record.

Detection and Quantification of Endotoxin in Blood

We employed a recently developed assay for the rapid detection of circulating endotoxin (SRE).[17,18] The active agent of this assay is a chemical conjugate of a monoclonal antibody that binds to the RBC surface (but itself does not cause agglutination) and a cyclic peptide antibiotic, polymyxin B. On mixing the test reagent with a blood sample, the antibody-polymyxin B conjugate will coat the RBCs. Endotoxin binds to the antibody-polymyxin B conjugate causing cross-linking between RBCs that results in visible agglutination in the presence of circulating endotoxin. All SRE assays were interpreted by a single laboratory technician blinded to the clinical data. For each test sample 10 μL of whole blood was pipetted into a reaction well on an agglutination tray. One drop of test reagent was added to the blood sample and mixed using gentle rocking of the agglutination tray for 2 min. A result was positive if any agglutination was detected compared with a negative control well.

We employed an LAL assay to quantify the concentration of endotoxin within blood using a commercial test kit (Limulus

Ameobocyte Lysate Pyrochrome; Associates of Cape Cod Inc; Woods Hole, Mass). The end point chromogenic method with diazo-coupling was employed as described by van Deventer and colleagues.[19] Blood specimens (4 to 5 mL) were collected in sterile nonpyrogenic tubes containing 120 IU of sodium heparin (Endotube-Chromogenix) and freshly prepared. No endotoxin was detected in empty tubes filled with pyrogen-free water. All samples were centrifuged (150g for 10 min at 4°C) and platelet-rich plasma was separated. Inhibiting and enhancing components were eliminated by dilution (10-fold in pyrogen-free water) and heating (10 min at 75°C) before the assay was performed. Platelet-rich plasma (0.1 mL) was added to the reaction vessel along with Pyrochrome to give a 1:1 ratio after which the samples were mixed on a plate shaker for 30 s prior to being incubated for 40 min at 37°C. The plates were removed from the incubator and the reaction was stopped with 0.05 mL of HCl-reconstituted sodium nitrite. Reconstituted ammonium sulfamate (0.05 mL) was then added to each plate after which 0.05 mL of n-(I-naphthyl)-ethylenediamine was added. Almost immediately, a magenta color developed and after 3 min, full colorization occurred, at which time the test was read at 490 nm.

In the presence of endotoxin, factors in LAL are activated in a proteolytic cascade resulting in the cleavage of a colorless artificial peptide substrate present in the pyrochrome LAL. Proteolytic cleavage of this substrate liberates p-nitroaniline, which undergoes reaction to form a diazotized magenta derivative that absorbs light at 490 nm. A standard curve, consisting of measured optical density plotted against known standard endotoxin concentrations, was used to determine the endotoxin concentrations in the blood specimens. The limit of detection of the assay was 0.005 endotoxin units (EU) per milliliter. All assays were performed in duplicate and adhered to Food and Drug Administration guidelines on the performance of the LAL test.[20]

Statistical Analysis

The analytical approach regarding the statistics was prospectively determined to detect relationships between patient outcomes and measurements of endotoxin. All comparisons were unpaired and all tests of significance were two tailed. Continuous variables were compared using analysis of variance for normally distributed variables and the Kruskal-Wallis test for nonparametrically distributed variables. The χ^2 statistic or Fisher's Exact Test was used to compare categorical variables. The area under receiver operating characteristic (ROC) curves was measured in a standard manner.[21] ROC curve analysis was used to determine the optimal threshold concentration of circulating endotoxin predictive of the development of multiorgan dysfunction.

We performed logistic regression analysis[22] using a commercial statistical package.[23] All continuous and ordinal independent variables were dichotomized based on clinically relevant subdivisions using the results of univariate analysis. Study variables with $p<0.20$ were included in the multivariate models and entered as categorical variables (*ie*, dummy variables), taking a value of 1 to indicate that a factor was present and 0 to indicate its absence.[24] A stepwise approach was used for entering new terms into the models with 0.05 as the limit for their acceptance or removal. The multivariate analyses were performed using models that were judged *a priori* to be clinically sound. This was prospectively determined to be necessary to avoid producing spuriously significant results with multiple comparisons.[22] Odds ratios were computed from the coefficients and 95% confidence intervals (CIs) were calculated for significant variables. Model over fitting was examined by evaluating the ratio of outcome events to the total number of independent variables in the final model, and specific testing for interactions among all significant predictor variables was included in our analyses.[25]

RESULTS

Patient Demographics

A total of 265 consecutive patients requiring medical ICU admission were evaluated. The mean age of the study population was 58.5±18.5 years and 47.2% were female. Sixty patients (22.6%) developed multiorgan dysfunction during their ICU stay defined as an OSFI >2. Baseline demographic information and hospital mortality according to the development of multiorgan dysfunction are shown in Table 1. Patients developing multiorgan dysfunction were significantly more likely to be male ($p=0.032$), were more often white ($p=0.035$), had a greater severity of illness as assessed by APACHE II ($p<0.001$), were more likely to have an ICU admission diagnosis of sepsis, ARDS, renal failure, or cardiac arrest, and were less likely to have an ICU admission diagnosis of asthma/COPD, drug overdose, pneumonia, GI hemorrhage, primary pulmonary hypertension, or pulmonary embolism compared with patients who did not develop multiorgan dysfunction (Table 1).

Endotoxin Detection and Quantitative Measurements

Among all study patients, the mean value for the concentration of circulating endotoxin as measured

Table 1—*Baseline Characteristics and Hospital Mortality*

Variable*	Multiorgan Dysfunction: Present (n=60)	Multiorgan Dysfunction: Absent (n=205)	p Value
Age, yr	58.7±17.9	58.4±18.7	0.601
Sex, No. (%)			
Male	39 (65.0)	101 (49.3)	0.032
Female	21 (35.0)	104 (50.7)	
Race, No. (%)			
White	37 (61.7)	101 (49.3)	0.035
Black	22 (36.7)	104 (50.7)	
Other	1 (1.6)	0 (0.0)	
APACHE II score	23.8±7.7	14.9±6.8	<0.001
Ratio of PaO_2 to FIO_2	281±137	286±153	0.846
Diagnoses, No. (%)			
Asthma/COPD	4 (6.7)	35 (17.1)	0.003
PPH	0 (0.0)	4 (1.9)	
Sepsis	18 (30.0)	32 (15.6)	
Drug overdose	2 (3.3)	19 (9.3)	
Congestive heart failure	3 (5.0)	9 (4.4)	
ARDS	2 (3.3)	0 (0.0)	
GI hemorrhage	10 (16.7)	42 (20.5)	
Unstable angina/MI	2 (3.3)	9 (4.4)	
Renal failure	2 (3.3)	4 (1.9)	
Pneumonia	6 (10.0)	27 (13.2)	
Pulmonary embolism	0 (0.0)	8 (3.9)	
Cardiac arrest	5 (8.3)	3 (1.5)	
Miscellaneous	6 (10.0)	13 (6.3)	
Hospital mortality, No. (%)	33 (55.0)	13 (6.3)	<0.001

*PPH=primary pulmonary hypertension; MI=myocardial infarction.

by the LAL assay was 3.7±3.5 EU/mL (range, 0 to 20.3 EU/mL). ROC curve analysis demonstrated that an endotoxin concentration >5 EU/mL had the best operating characteristics for the prediction of multiorgan dysfunction on ICU days 1 and 2. The concentration of circulating endotoxin was greater for patients developing multiorgan dysfunction compared with patients who did not develop multiorgan dysfunction on ICU day 1 (4.3±4.5 EU/mL vs 3.6±3.2 EU/mL; p=0.137) and on ICU day 2 (4.4±3.8 EU/mL vs 3.4±3.0 EU/mL; p=0.053).

We found that the development of multiorgan dysfunction was significantly greater among SRE-positive patients (44.8%) compared with SRE-negative patients (21.9%) on ICU day 2 (p=0.013) (Table 2). Patients with a positive SRE had circulating concentrations of endotoxin that were greater than those observed in patients with a negative SRE on ICU day 1 (4.1±3.5 EU/mL vs 3.6±3.5 EU/mL; p=0.137) and on ICU day 2 (4.4±3.9 EU/mL vs 3.4±3.0 EU/mL; p=0.127). No statistically significant relationship was observed between the measurement of circulating endotoxin and the development of Gram-negative infection, severe sepsis, or septic shock (p>0.20).

The concentration of endotoxin between hospital survivors and nonsurvivors on ICU day 1 (3.6±3.2 EU/mL vs 4.4±4.6 EU/mL; p=0.913) and on ICU day 2 (3.6±3.1 EU/mL vs 3.9±3.8 EU/mL; p=0.639) were not statistically different. Similarly, the mortality rate for SRE-positive patients was not statistically different from the mortality rate for SRE-negative patients on ICU day 1 (12.7% vs 18.6%; p=0.308) and ICU day 2 (27.6% vs 14.9%; p=0.109).

Multiorgan Dysfunction as an Outcome

Table 3 shows the 12 characteristics qualifying for multivariate analysis on ICU day 1. Multivariate analysis identified the presence of severe sepsis or septic shock (adjusted odds ratio, 1.8; 95% CI, 1.5 to 2.1; p<0.001) and an APACHE II score >20 (adjusted odds ratio, 1.1; 95% CI, 1.1 to 1.2; p<0.001) as being independently associated with the occurrence of multiorgan dysfunction. A similar analysis was performed for the patients remaining in the ICU on day 2 (Table 4). Multivariate analysis demonstrated independent associations between the development of multiorgan dysfunction and the presence of a positive SRE assay, the occurrence of severe sepsis or septic shock, and having an APACHE II score >20 (Table 5). For patients in the ICU after day 2, three variables were independently associated with the development of multiorgan dysfunction: having infection due to Gram-negative bacteria (adjusted odds ratio, 3.4; 95% CI, 2.1 to 5.6; p=0.014), the presence of severe sepsis or septic shock (adjusted odds ratio, 1.7; 95% CI, 1.3 to 2.2; p=0.048), and having an APACHE II score >20 (adjusted odds ratio, 1.1; 95% CI, 1.1 to 1.2; p=0.002).

Table 2—*Multiorgan Dysfunction and the Detection of Circulating Endotoxin Using SRE*

Day Patients Present in ICU	Development of Multiorgan Dysfunction (%)	p Value
ICU day 1		
SRE-positive	12/55 (21.8)	0.870
SRE-negative	48/210 (22.9)	
ICU day 2		
SRE-positive	13/29 (44.8)	0.013
SRE-negative	25/114 (21.9)	
ICU day >2		
SRE-positive	14/30 (46.7)	0.089
SRE-negative	28/94 (29.8)	

ICU Mortality as an Outcome

Patients developing multiorgan dysfunction had a significantly greater hospital mortality rate compared to patients without multiorgan dysfunction (relative risk, 8.7; 95% CI, 4.9 to 15.4) (Table 1). Multivariate analysis of patients in the ICU on day 1 demonstrated that multiorgan dysfunction (adjusted odds ratio, 11.2; 95% CI, 7.4 to 17.0; p<0.001) and the presence of severe sepsis or septic shock (adjusted odds ratio, 1.7; 95% CI, 1.4 to 2.0; p=0.004) were independently associated with hospital mortality. A similar analysis of the 143 patients remaining in the ICU on day 2 found the same two variables to be independently associated with hospital mortality (multiorgan dysfunction [adjusted odds ratio, 10.2; 95% CI, 5.9 to 17.8; p<0.001]; presence of severe sepsis or septic shock [adjusted odds ratio, 1.8; 95% CI, 1.4 to 2.3; p=0.018]). Neither the bedside SRE assay nor the LAL assay predicted hospital mortality.

Lengths of Stay

Patients developing multiorgan dysfunction had significantly longer lengths of stay in the ICU (6.8±8.9 days vs 2.6±2.6 days; p<0.001) and the hospital (22.6±23.9 days vs 9.4±8.6 days; p<0.001) compared to patients without multiorgan dysfunction. No significant differences were observed in lengths of stay according to either the SRE or LAL assays for ICU days 1 and 2 (p>0.10). However, patients with a positive SRE assay after ICU day 2 (n=30) had significantly longer lengths of stay in the ICU (8.4±9.0 days vs 5.2±5.8 days; p=0.029) and longer lengths of hospitalization (26.4±26.8 days vs

Table 3—*Characteristics Evaluated for Independent Association With Multiorgan Dysfunction on ICU Day 1*

Characteristic	Multiorgan Dysfunction: Present (n=60)	Multiorgan Dysfunction: Absent (n=205)	Odds Ratio	p Value
Intra-abdominal infection				
Yes	6 (10.0)	1 (0.5)	22.7	<0.001
No	54 (90.0)	204 (99.5)		
APACHE II score				
>20	39 (65.0)	39 (19.0)	7.9	<0.001
≤20	21 (35.0)	166 (81.0)		
Severe sepsis or septic shock				
Yes	35 (58.3)	40 (19.5)	5.8	<0.001
No	25 (41.7)	165 (80.5)		
Gram-negative bacterial infection				
Yes	26 (43.3)	36 (17.6)	3.6	<0.001
No	34 (56.7)	169 (82.4)		
Bacteremia				
Yes	17 (28.3)	21 (10.2)	3.5	<0.001
No	43 (71.7)	184 (89.8)		
Gram-positive bacterial infection				
Yes	19 (31.7)	32 (15.6)	2.5	0.006
No	41 (68.3)	173 (84.4)		
Fungal infection				
Yes	10 (16.7)	15 (7.3)	2.5	0.029
No	50 (83.3)	190 (92.7)		
Urinary tract infection				
Yes	19 (31.7)	33 (16.1)	2.4	0.008
No	41 (68.3)	172 (83.9)		
Pneumonia				
Yes	24 (40.0)	52 (25.4)	2.0	0.027
No	36 (60.0)	153 (74.6)		
Sex				
Male	39 (65.0)	101 (49.3)	1.9	0.032
Female	21 (35.0)	104 (50.7)		
Race				
White	37 (61.7)	101 (49.3)	1.7	0.091
Nonwhite	23 (38.3)	104 (50.7)		
LAL assay, EU/mL				
>5	16 (26.7)	36 (17.6)	1.7	0.123
≤5	44 (73.3)	169 (82.4)		

14.6±9.9 days; p=0.001) compared to patients who did not develop a positive SRE after ICU day 2 (n=94).

Discussion

This preliminary study confirms the importance of multiorgan dysfunction as a determinant of mortality among medical ICU patients. The definition of multiorgan dysfunction we used was based on our prior institutional experience demonstrating that a threshold value for the OSFI of >2 yielded the best operating characteristics for the prediction of hospital mortality and prolonged hospital lengths of stay.[3,16] We also showed that a rapid qualitative assay for the detection of circulating endotoxin (SRE) is predictive of the development of multiorgan dysfunction. The SRE assay has been demonstrated to be sensitive for the detection of various species of endotoxin *in vitro* and for the detection of circulating endotoxin *in vivo* (lower limit of circulating endotoxin detection *in vivo*: 1.2 EU/mL).[17,18] The SRE assay has also been shown to have good specificity *in vivo*, with a false-positive rate among healthy blood bank control samples of 1.5%.[18] Lastly, we found that the SRE assay was superior to the LAL assay for the prediction of multiorgan dysfunction among critically ill medical patients.

The incidence of sepsis has been increasing and its occurrence is associated with a mortality varying from 20 to 60% among ICU patients.[4,26] This has led to the development and investigation of various antiendotoxins and anticytokines aimed at ameliorating or reversing the host's response to sepsis to

Table 4—*Characteristics Evaluated for Independent Association With Multiorgan Dysfunction on ICU Day 2*

Characteristic	Multiorgan Dysfunction: Present (n=38)	Multiorgan Dysfunction: Absent (n=105)	Odds Ratio	p Value
Intra-abdominal infection				
Yes	5 (13.2)	1 (1.0)	15.8	0.005
No	33 (86.8)	104 (99.0)		
APACHE II score				
>20	24 (63.2)	22 (21.0)	6.5	<0.001
≤20	14 (36.8)	83 (79.0)		
Severe sepsis or septic shock				
Yes	20 (52.6)	27 (25.7)	3.2	0.002
No	18 (47.4)	78 (74.3)		
SRE				
Positive	13 (34.2)	16 (15.2)	2.9	0.013
Negative	25 (65.8)	89 (84.8)		
Bacteremia				
Yes	11 (28.9)	15 (14.3)	2.4	0.045
No	27 (71.1)	90 (85.7)		
Gram-negative bacterial infection				
Yes	16 (42.1)	25 (23.8)	2.3	0.033
No	22 (57.9)	80 (76.2)		
Fungal infection				
Yes	8 (21.1)	11 (10.5)	2.3	0.100
No	30 (78.9)	94 (89.5)		
Sex				
Male	25 (65.8)	53 (50.5)	1.9	0.104
Female	13 (34.2)	52 (49.5)		
Gram-positive bacterial infection				
Yes	13 (34.2)	23 (21.9)	1.9	0.134
No	25 (65.8)	82 (78.1)		
LAL assay,* EU/mL				
>5	11 (29.7)	18 (17.5)	2.0	0.115
≤5	26 (70.3)	85 (82.5)		

*The LAL assay was not performed in three patients on ICU day 2.

reduce end-organ dysfunction and mortality.[8] To date, and to our knowledge, none of these therapies has been shown to be effective in altering patient outcomes. In part, this may be due to the heterogeneous patient populations often examined in these investigations. This has resulted in numerous clinical commentaries offering explanations for the negative results of these trials and suggestions for ways in which future investigations could be improved.[27-30] A common theme of these communications has been the need for a more precise classification of sepsis, so that patients likely to respond to new treatments can be more accurately identified.

Table 5—*Multivariate Logistic-Regression Analysis of Characteristics Present on ICU Day 2 Independently Associated With Multiorgan Dysfunction*

Characteristic	Adjusted Odds Ratio	95% CI	p Value
Positive SRE	4.1	2.5-6.8	0.006
Severe sepsis or septic shock	1.6	1.3-2.1	0.029
APACHE II score >20	1.2	1.1-1.2	<0.001

Patients with sepsis are often included in clinical trials of new therapies before microbiological documentation of infection is obtained. This is thought to be necessary to provide the therapy prior to the development of irreversible end-organ dysfunction.[4] Identifying patients with endotoxemia has been suggested as a more objective means of targeting patients who would be likely to respond to various antiendotoxin and anticytokine therapies. Casey and coworkers[10] demonstrated that endotoxin and other circulating cytokines were present at higher levels in patients with sepsis compared to critically ill patients without sepsis. These authors also demonstrated that an endotoxin-cytokine score could be derived that was closely associated with mortality.[10] However, other investigators have failed to demonstrate any relationship between the presence of endotoxemia and hospital mortality.[13,31]

There are several potential explanations for the discrepancies observed among studies attempting to

link endotoxin measurements to clinical outcomes. First, the presence of endotoxemia may be insufficient to result in patient mortality unless it is associated with end-organ dysfunction. This is suggested by studies demonstrating that antiendotoxin therapies directed at patients with Gram-negative sepsis and organ failure experienced greater resolution of organ failure compared to patients receiving placebo.[32] Second, endotoxin may primarily result in morbidity and end-organ dysfunction among hosts with ineffective endogenous antiendotoxin defenses.[33] Third, our basic knowledge of the complex timing of mediator release and balance during sepsis may be insufficient to completely understand the relationship of circulating endotoxin (and other cytokines) to patient outcomes.[9] Targeting a single microbial toxin (*eg*, endotoxin) may not represent a viable strategy for treating a complex inflammatory response to diverse Gram-negative bacteria.[8] However, the ability to rapidly identify a group of patients at higher risk for multiorgan dysfunction could allow for more homogeneous study populations to be examined in clinical trials. This would help to standardize the evaluation of new sepsis therapies among patients with similar outcomes.[34]

Our study has several limitations. First, we evaluated all admissions to the medical ICU. Other investigators have demonstrated that targeting high-risk patients, such as those with severe sepsis or septic shock, will result in a higher incidence of multiorgan dysfunction.[4,26] Second, we examined only medical patients, many of whom were in the ICU for high-risk monitoring and were discharged within 24 h. Therefore, these results may not be applicable to nonmedical patients. Additionally, our patient mix may explain the discrepancy we observed between ICU days 1 and 2. For example, most patients (80.8%) with GI hemorrhage did not develop multiorgan dysfunction and required intensive care monitoring for ≤24 h. However, 38.1% of these patients had a positive SRE assay, probably related to GI translocation of endotoxin.

The final limitation of our study has to do with the endotoxin assays themselves. The LAL assay measures only heat-extracted endotoxin.[19] This makes the interpretation of the results obtained with the LAL assay problematic since individual endotoxin species may have different circulating half-lives and different spectrums of binding proteins.[35] Similarly, not all endotoxin preparations cause agglutination to occur with the SRE assay.[17] This may be explained, in part, by the physical state of the endotoxin preparation (*eg*, formation of micelles) or the protein binding of endotoxin which may also interfere with the agglutination reaction.[17,18] At present, it is not clear in what forms endotoxin circulates in the bloodstream and to what degree variation exists among these forms. Therefore, assays such as the LAL and SRE may be measuring different forms of circulating endotoxin accounting for the discrepant results we observed with these two assays.

In summary, this study suggests that a rapid qualitative assay for the detection of circulating endotoxin is predictive of the development of multiorgan dysfunction. We also found that a positive SRE assay was associated with higher concentrations of circulating endotoxin. The independent association of a positive SRE with multiorgan dysfunction suggests that this assay may be useful to target high-risk patients for future clinical investigations. Further studies are planned to validate these results, identify potential mechanisms to explain the predicted differences in outcome based on different endotoxin assays, and to examine the predictive accuracy of the SRE assay for specific subgroups of ICU patients.

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