MAJOR ARTICLE

Diagnostic and Prognostic Implications of Endotoxemia in Critical Illness: Results of the MEDIC Study

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A novel assay for endotoxin, based on the ability of antigen-antibody complexes to prime neutrophils for an augmented respiratory burst response, was studied in a cohort study of 857 patients admitted to an intensive-care unit (ICU). On the day of ICU admission, 57.2% of patients had either intermediate (≥0.40 endotoxin activity [EA] units) or high (≥0.60 units) EA levels. Gram-negative infection was present in 1.4% of patients with low EA levels, 4.9% with intermediate levels, and 6.9% with high levels; EA had a sensitivity of 85.3% and a specificity of 44.0% for the diagnosis of gram-negative infection. Rates of severe sepsis were 4.9%, 9.2%, and 13.2%, and ICU mortality was 10.9%, 13.2%, and 16.8% for patients with low, intermediate, and high EA levels, respectively. Stepwise logistic regression analysis showed that elevated Acute Physiology and Chronic Health Evaluation II score, gram-negative infection, and emergency admission status were independent predictors of EA.

Endotoxin, a complex lipopolysaccharide (LPS) that is present in the cell walls of gram-negative bacteria, is a potent trigger of innate immunity [1]. In vitro exposure of cells of the innate immune system to endotoxin results in changes in the expression of >300 genes [2, 3], whereas its systemic administration in experimental animals leads to the activation of macrophages, neutrophils, endothelial cells, and the coagulation cascade and to the release of a complex cascade of host-derived inflammatory mediators [4]. Virtually all of the physiological and biochemical derangements of septic shock, including organ failure and acute lethality, can be reproduced in experimental animals by the systemic administration of very small amounts of endotoxin [5, 6]. The administration of nanogram quantities of endotoxin to human volunteers reliably evokes the clinical and biochemical features of acute systemic inflammation [7], whereas larger amounts produce septic shock and multiple organ dysfunction [8]. Moreover, endotoxin alone can produce the wide range of manifestations of infection with viable gram-negative organisms [9].

By weight, endotoxin makes up ~10% of the cell wall of a gram-negative bacterium; thus, infection with viable gram-negative bacteria can result in significant exposure to endotoxin. However, studies of the prevalence of endotoxemia in cohorts of critically ill patients have shown it to occur much more commonly than culture-proven gram-negative infection [10–12]. Because of the predominance of gram-negative species in the normal intestinal flora, the gastrointestinal tract is an important

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reservoir, containing as much as 25 g of endotoxin [13], and gut endotoxin has been implicated as a source of endotoxemia after vigorous exercise [14]. Exposure to endotoxin can also occur through environmental exposure—for example, in cigarette smoke [15] and during mechanical ventilation [16].

Although endotoxin is ubiquitous, it has been notoriously difficult to measure reliably in human illness. The most commonly used diagnostic test—the chromogenic limulus amebocyte lysate assay [17]—is based on the ability of endotoxin to induce coagulation of the hemolymph of the horseshoe crab, *Limulus polyphemus* [18]. The assay has been widely used to detect endotoxin contamination of drugs and fluids; however, its utility in biological samples has been limited [19], because of circulating inhibitors of the coagulation reaction. Moreover, other microbial products, notably from fungi, can activate the limulus reaction, so the assay is not specific for endotoxin.

Gram-negative infection is one of the many infectious causes of sepsis, a life-threatening disorder that results from the activation of the innate immune system. Yet culture-proven gram-negative infection is documented in only a minority of patients with sepsis [20]. We recently described a novel assay methodology that can detect endotoxin in whole blood by use of neutrophil-dependent chemiluminescence [21]. We therefore sought to define the prevalence of endotoxemia in a population of critically ill patients, to determine its association with invasive infection and to assess the correlation of endotoxemia at the time of admission to an intensive-care unit (ICU) with sepsis and ICU outcome.

SUBJECTS AND METHODS

Study population. We undertook a prospective observational cohort study of critically ill patients admitted to 1 of 10 ICUs in Canada, the United States, Belgium, and England. Patients were excluded if they had known von Willebrand's disease, were undergoing plasmapheresis, had received >3 U of red blood cells during the preceding 6 h, or were enrolled in a clinical trial of an antiendotoxin therapy. Unless the local institutional review board explicitly waived the need for written, informed consent for a single baseline determination of endotoxin levels, consent was obtained from each patient or a surrogate decision maker. We enrolled consecutively admitted patients as possible, subject to the need for informed consent and the availability of resources to perform the assay on weekends; the first patient was recruited in January 2000 and the last in September 2000. All patients were studied during the first 24 h of ICU admission.

We recruited 97 healthy ambulatory volunteers from the sponsor's manufacturing facility to establish normal levels for the endotoxin activity (EA) assay. Each volunteer provided informed consent.

Diagnosis of infection and severe sepsis. Episodes of infection were diagnosed by microbiologic, laboratory, radiologic, and operative data, using criteria modified from those of the Centers for Disease Control and Prevention (CDC) [22]. Cases that met CDC criteria for infection were further reviewed by a clinical evaluation committee (CEC), whose members were blinded to the EA data. Each case was reviewed by 2 fellows and ⩾1 senior intensivist with expertise in infections in the ICU; when disagreements in the adjudication process arose, cases were further reviewed by a second senior intensivist. If necessary, a final adjudication was reached through a consensus process that involved the entire committee.

Severe sepsis was considered to be present when criteria for sepsis syndrome [20, 23] were met: temperature >38.3°C or <35.6°C, respiratory rate >20/min or need for mechanical ventilation, heart rate >90 beats/min, and clinical evidence of infection in association with ⩾1 of the following manifestations

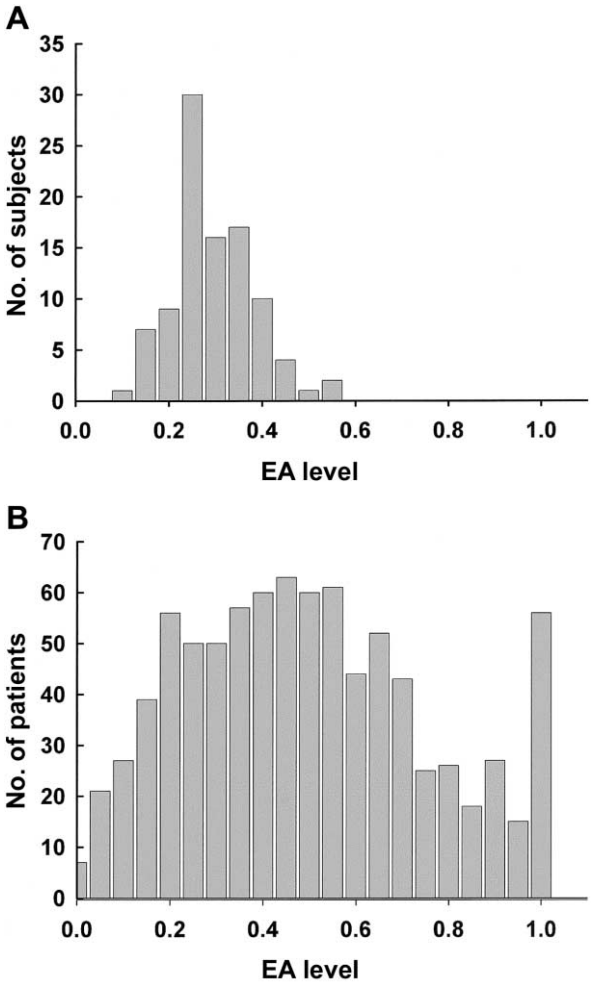

Figure 1. Endotoxin activity (EA) in blood from healthy volunteers and from critically ill patients in the intensive care unit (ICU). *A,* EA in whole-blood samples obtained from 97 healthy volunteers. Low-level activity was evident in the majority of subjects, although in none was the level >0.60 EA units. *B,* Distribution of EA levels in the 857 patients studied, on the day of their admission to the ICU.

Table 1. Demographic characteristics of the study population.

Characteristic	Value
Age, years (IQR)	60.2 ± 17.2 (49, 74)
Male, %	58.9
Nonwhite, %	15.8
Geographic origin, % (no.)	
Europe	42.5 (364)
Canada	40.0 (343)
United States	17.5 (150)
Admission severity of illness score, mean ± SD (IQR)	
APACHE II	15.2 ± 9.5 (8, 21)
MOD	3.9 ± 3.4 (1, 6)
SOFA	4.9 ± 3.9 (2, 7)
Diagnosis at admission	
Infection/sepsis	88
MI/CHF/cardiac arrest	120
Gastrointestinal/hepatic infection	28
Multiple trauma	31
Metabolic/endocrine illness	14
Neurological	95
Major elective surgery	273
Respiratory failure	128
Organ transplant	34
Other	46
Medical admission, no. (%)	450 (52.5)
Surgical admission, no. (%)	407 (47.5)
ICU length of stay, days	
Mean ± SD (IQR)	5.4 ± 10.9 (2, 6)
Median	2
Hospital length of stay, days	
Mean ± SD (IQR)	23.3 ± 27.0 (7, 29)
Median	14
Mortality, no. (%)	
ICU	114 (13.3)
Hospital	170 (19.9)

NOTE. CHF, congestive heart failure; ICU, intensive-care unit; IQR, interquartile range (25th percentile, 75th percentile); MI, myocardial infarction; MOD, Multiple Organ Dysfunction; SOFA, Sequential Organ Failure Assessment.

of organ dysfunction: PaO_2:FIO_2 <280 mm Hg, pH <7.30, urine output <720 mL/24 h, and mean arterial pressure <80 mm Hg. Shock was defined as a mean arterial pressure <60 mm Hg or the use of vasopressor therapy (other than dopamine at a dose of $\leqslant 5$ μg/kg).

Severity of illness measurements. Baseline severity of illness was quantified using the APACHE II score [24]; the degree of baseline organ dysfunction was quantified using the Multiple Organ Dysfunction (MOD) [25] and Sequential Organ Failure Assessment (SOFA) [26] scores.

Chemiluminescent assay for endotoxin. EA in whole blood was measured as described elsewhere [21], by use of a murine IgM monoclonal antibody raised against the lipid A of *Escherichia coli* J5. This antibody is broadly cross-reactive against gram-negative bacteria but only weakly cross-reactive against *Bacteroides* species; it does not cross-react with gram-positive bacteria [21]. Samples of 40 μL of whole blood were incubated in duplicate with saturating concentrations of antibody, then stimulated with zymosan. The resulting respiratory burst activity was detected as light release from the lumiphor luminol, by use of a chemiluminometer (Autolumat LB953; E.G. & G. Berthold). The LPS/anti-LPS complex primes the patient's neutrophils for an augmented response to stimulation with zymosan; by measuring basal (no antibody) and maximally stimulated (4600 pg/mL LPS) responses in the same blood sample, the EA of the test specimen can be calculated by integrating the chemiluminescence over time. Levels are expressed as EA units and represent the mean of duplicate determinations from the same sample. EA is expressed in relative units derived from the integral of the basal and stimulated chemiluminescent response. An EA level of 0.4 is approximately equivalent to an endotoxin concentration of 25–50 pg/mL, and a level of 0.6 is approximately equivalent to an LPS concentration of 100–200 pg/mL of *E. coli* 055:B5 LPS. Because the assay detects the lipid A component of LPS, variations in chain length and lipid A structure of LPS will affect the relationship between EA and the mass of LPS.

Statistical analysis. The 2-sample Student's *t* test or Wil-

Table 2. Sites and microbiological analysis of infections in study cohort.

Site, infecting organism	Infection, by criteria	
	CDC	CEC
Bloodstream		
Gram-negative		
Escherichia coli	2	2
Pseudomonas aeruginosa	1	1
Other	1	1
Gram-positive		
Coagulase-negative *Staphylococcus*	5	2
Staphyloccocus aureus	6	6
Enterococcus	2	2
Streptococcus pneumoniae	0	1
Other	1	1
Fungi		
Candida	1	1
Lung		
Gram-negative		
Haemophilus influenzae	3	2
P. aeruginosa	3	1
Klebsiella	4	3
E. coli	9	3
Other	5	1
Gram-positive		
S. aureus	10	4
S. pneumoniae	4	4
Other	2	2
Fungi		
Candida	7	1
Abdomen		
Gram-negative		
E. coli	2	2
Enterobacter	2	1
Other	0	2
Gram-positive		
Enterococcus	4	3
S. aureus	2	2
Clostridium species	1	1
Other	1	1
Fungi		
Candida	1	1
Urinary tract		
Gram-negative		
E. coli	2	0
Other	2	0
Gram-positive		
Streptococci	2	2
Enterococcus	4	0
Fungi		
Candida	1	0
Skin/soft tissue		
Gram-negative	3	1
Gram-positive	7	7
Superficial surgical site		
Gram-negative	2	0
Gram-positive	4	1
Central nervous system		
Gram-negative		
Klebsiella	1	1

NOTE. CDC, Centers for Disease Control and Prevention; CEC, clinical evaluation committee.

coxon–Mann-Whitney *U* test was used to compare continuous variables. Categorical variables were evaluated by use of Pearson's χ^2 test, Fisher's exact test, or the Mantel-Haenzel χ^2 test, as appropriate. The association between adverse outcome and EA level was modeled using logistic regression analysis and is reported as estimated odds ratio and 95% confidence interval (CI). A multivariate logistic regression model was used to estimate the association between endotoxemia and severe sepsis, adjusting for covariates (age, sex, presence of systemic inflammatory response syndrome [SIRS], and APACHE II score). Normally distributed data are presented as mean ± SD. Statistical significance was assumed for values of $P < .05$.

RESULTS

Establishment of cutoffs for EA. EA was measured in a population of 97 healthy volunteers, to establish normal EA levels. The median EA level in this population was 0.26 EA units (figure 1*A*). Of the 97 samples, EA levels were <0.40 in 90 (93%) patients and between 0.40 and 0.60 in the remaining 7 (7%) patients; no healthy volunteer had a level >0.60. Thus, EA levels of 0.40 and 0.60 represented increments of >2 and 4 SDs from this normal value, respectively, and results are aggregated as low (<0.40 EA units), intermediate (0.40–0.60 EA units), and high (≥0.60 EA units). We measured EA in a separate pilot study of 146 patients in the ICU in 3 of the participating sites and found that these cutoffs yielded patient groups of roughly comparable size, with 44% of patients having low, 31% intermediate, and 25% high EA levels.

Patient demographics. We measured EA levels in 857 of 1010 patients screened for study enrollment on the day of their ICU admission, between January and September 2000; demographic data for these 857 patients are summarized in table 1. The remaining 153 patients were excluded because of lack of informed consent ($n = 53$) or a nonevaluable EA assay result ($n = 100$). Assay results could not be evaluated in this latter group of patients (9.9% of the screened population) for 1 of 4 reasons. For 61 patients, either the negative or positive control yielded discordant results, rendering the calculation of the sample value unreliable; samples from a further 6 patients failed to respond to the positive endotoxin control. Instrument or software malfunction occurred during 6 assays, and a further 27 specimens were not analyzed in the lab in a timely fashion. The demographic characteristics of these nonevaluable patients did not differ significantly from those of the reported cohort.

Prevalence, site, and microbiological results of infections in the study cohort. Using CDC criteria, infection was determined to be present on the day of ICU admission in 73 patients (8.5% of the study cohort); review by the CEC resulted in 27 of these cases being reclassified as colonization rather than true invasive infection and in a further 3 cases (which did not meet CDC criteria) being reclassified as infected, so that the prevalence of infection on the first ICU day using CEC criteria was 5.7% (49 patients). The CEC evaluation resulted in reclassification of 26 of 47 microbial isolates from cases of pneumonia, 9 of 11 isolates from cases of urinary tract infection, and 7 of

Table 3. Endotoxin activity (EA) level and the prevalence of infection (by Centers for Disease Control and Prevention criteria).

	Gram-negative infection[a]		Gram-positive infection[b]		All infections[c]	
EA level	Prevalence, % (no./total)	OR (95% CI)	Prevalence, % (no./total)	OR (95% CI)	Prevalence, % (no./total)	OR (95% CI)
Low (<0.40)	1.4 (5/367)	…	3.8 (14/367)	…	5.2 (19/367)	…
Intermediate (0.40–0.60)	4.8 (11/228)	3.7 (1.3–10.7)	7.9 (18/228)	2.2 (1.1–4.4)	11.4 (26/228)	2.4 (1.3–4.4)
High (≥0.60)	6.9 (18/262)	5.3 (2.0–14.6)	5.7 (15/262)	1.5 (0.7–3.2)	10.7 (28/262)	2.2 (1.2–4.0)

NOTE. CI, confidence interval; OR, odds ratio.

[a] Mantel-Haenzel $\chi^2 = 12.538$, $P = .0004$.

[b] Mantel-Haenzel $\chi^2 = 1.429$, $P = .23$.

[c] Mantel-Haenzel $\chi^2 = 6.699$, $P = .0097$.

16 isolates from infections of the skin, soft tissues, or surgical wound; adjudications of bloodstream infection, intraabdominal infection, and central nervous system infection supported the CDC diagnosis in all cases, with the exception of the exclusion of 3 isolates of coagulase-negative staphylococci from blood cultures. Table 2 summarizes the sites and microbiology of these infections. Patients who were infected on the day of ICU admission were sicker, as reflected in higher APACHE II, MOD, and SOFA scores. Using CDC diagnostic criteria, infection at ICU admission was associated with an increased risk of ICU mortality (30.1% vs. 11.7%; $P < .001$); the mortality rate in patients diagnosed with infection by the CEC criteria (30.6% vs. 12.3% for those without infection) did not differ from that of patients diagnosed using CDC criteria.

High rate of endotoxemia in critically ill patients. Although microbiologically proven infection was relatively uncommon in the study cohort, endotoxemia was present on the day of ICU admission in the majority of patients. EA levels were <0.40 in 367 patients (42.8% of the population), between 0.40 and 0.60 in 228 patients (26.6%), and ≥0.60 in 262 patients (30.6%) (figure 1*B*); thus, the majority of patients in the study cohort had elevated endotoxin levels on the day of ICU admission.

Association of endotoxemia with both gram-negative and -positive infection. Infection with a gram-negative organism, diagnosed by use of criteria from the CDC, was present in 34 patients at the time of ICU admission (4.0% of the study cohort). Gram-negative infection was significantly more common in patients with endotoxemia, and the prevalence increased as the endotoxin level increased (table 3). Gram-positive infections were diagnosed in 47 patients (5.5%). Endotoxemia was weakly associated with gram-positive infection (table 3), although the prevalence of gram-positive infection was significantly increased only for patients with intermediate levels of EA. Intermediate or high levels of EA predicted a >2-fold increase in the odds of infection with any organism (table 3).

An EA level of <0.40 was associated with a low probability of infection. Only 5.2% of patients with an EA level of <0.40 had infection with any organism, and only 1.2% had an infection caused by a gram-negative organism. Endotoxemia, as measured by the chemiluminescent assay, had a sensitivity of 85.3% but a specificity of only 44.0% for the diagnosis of gram-negative infection; however, the negative predictive value (the ability of a low endotoxin level to exclude the diagnosis of gram-negative infection on the day of ICU admission) was 98.6% (95% CI, 97.5%–99.8%). Moreover, because endotoxemia was also more common in patients with gram-positive infection than in uninfected patients, the negative predictive value for endotoxemia to exclude any infection as defined by CDC criteria was 94.8% (95% CI, 92.6%–97.1%). Thus, although endotoxemia is common in critical illness, its absence can support the clinical conclusion that a critically ill patient is very unlikely to be infected.

Association of endotoxemia with severe sepsis. Criteria for severe sepsis were present at the time of admission in 74 patients (8.6% of the study cohort); ICU mortality for this population was higher than that of patients who did not have sepsis (32.4% vs. 11.5%; $P < .001$). EA levels were significantly higher for patients who met criteria for severe sepsis (0.57 ± 0.26 vs. 0.46 ± 0.26 units; $P < .001$), and the risk of severe sepsis increased with increasing increments of EA levels (table 4). Within the population of patients who had severe sepsis, however, increasing endotoxin levels did not predict an increased mortality risk. The results of multivariate regression analysis showed that, after controlling for age, sex, the presence of SIRS, and APACHE II score, increased EA at admission was signif-

Table 4. Endotoxin activity (EA) level and risk of severe sepsis.

EA level	Risk of severe sepsis in first 24 h of ICU admission, % (no./total)	OR (95% CI)[a]	*P*
Low (<0.40)	4.9 (18/367)	…	…
Intermediate (0.40–0.60)	9.2 (21/228)	2.0 (1.0–3.8)	<.05
High (>0.60)	13.4 (35/262)	3.0 (1.7–0.5)	<.001

NOTE. CI, confidence interval; ICU, intensive-care unit; OR, odds ratio.

[a] Mantel-Haenzel $\chi^2 = 13.962$, $P = .0002$.

Table 5. Endotoxin activity (EA) level and severity of illness at the time of hospital admission.

Test	EA level			Levels for trends of association, *P*
	Low (<0.40)	Intermediate (0.40–0.60)	High (⩾0.60)	
APACHE II, mean ± SD (IQR)	13.3 ± 8.7 (7.0, 17.0)	15.3 ± 9.6 (8.0, 21.0)[b]	17.6 ± 9.9 (10.0, 24.0)[a]	<.0001
Admission MOD, mean ± SD (IQR)	3.4 ± 3.2 (1, 5)	3.8 ± 3.3 (1.0, 5.3)	4.6 ± 3.6 (2.0, 7.0)[a]	.0001
Admission SOFA, mean ± SD (IQR)	4.3 ± 3.6 (2.0, 6.0)	4.9 ± 3.9 (2.0, 7.0)	5.7 ± 4.1 (3.0, 8.0)[a]	<.0001
Shock, %	11.6	20.5[b]	22.7[a]	.0004
PaO_2:FIO_2 ratio, mean ± SD (IQR)	253 ± 111 (185, 322)	215 ± 98 (145, 293)[a]	205 ± 102 (121, 280)[a]	<.0001
WBC <4 or >12 $\times 10^3$ cells/mm^3, %	41.9	48.1	56.5[a]	.0021

NOTE. IQR, interquartile range (25th percentile, 75th percentile); MOD, Multiple Organ Dysfunction; SOFA, Sequential Organ Failure Assessment; WBC, white blood cells.

[a] $P < .001$ vs. patients with admission EA levels <0.40.

[b] $P < .01$ vs. patients with admission EA levels <0.40.

icantly associated with the development of severe sepsis on the day of ICU admission, whether it was included as a continuous variable ($P = .02$) or as a categorical variable ($P < .0001$).

Correlation of endotoxemia with illness severity at the time of ICU admission. Because endotoxemia was detected much more frequently than culture-proven invasive gram-negative infection, we evaluated the association of endotoxin levels with noninfectious clinical variables. Patients with a higher EA level (⩾0.40 EA units) on the day of ICU admission were sicker, as reflected in higher severity indices (e.g., APACHE II score); they also manifested greater degrees of organ dysfunction, as reflected by shock and hypoxemia. White blood cell counts were more frequently abnormal, either increased or decreased, in patients with endotoxemia (table 5). Using stepwise logistic regression analysis, independent predictors of an elevated endotoxin level included acute severity of illness, as reflected in the APACHE II score ($P < .0001$), gram-negative infection diagnosed by use of CDC criteria ($P < .001$), and emergency admission to the ICU ($P < .05$).

Adverse outcome predicted by endotoxemia at the time of ICU admission. Patients with endotoxemia had increased ICU and hospital mortality (figure 2). The ICU length of stay was modestly longer for patients with EA levels ⩾0.60 at the time of ICU admission (6.8 ± 12.2 vs. 4.9 ± 10.7 days; $P < .05$).

DISCUSSION

Endotoxin is ubiquitous in the natural environment. A highly conserved component of the gram-negative bacterial cell wall, its importance to mammals is underlined by the complexity of the endogenous mechanisms that have evolved to recognize and respond to the molecule [9]. A distinct carrier protein, LPS-binding protein, transports circulating endotoxin and facilitates its recognition by the cell through a unique receptor, CD14; an associated pattern-recognition receptor, Tlr4; and an accessory protein, MD2, that transduces the endotoxin signal to the cell nucleus, leading to the expression of a complex network of inflammatory mediators [1]. These host-derived mediators have been centrally implicated in the pathogenesis of the clinical syndrome of sepsis, and neutralization of the activity of more than a dozen of these molecules can prevent endotoxin-mediated lethality [27].

However, endotoxin can also complex with lipoproteins such as high-density liver protein and with soluble CD14, and its activity in vivo is antagonized by bactericidal permeability–increasing protein and by the enzyme alkaline phosphatase [28]. The complexity of the biological mechanisms involved in the recognition of and response to bacterial endotoxin are complex, and defining its role in human illness has proved remarkably difficult. Furthermore, the available methods to detect endotoxin in biological fluids have been unreliable, so the study of the epidemiology of endotoxemia has been challenging. The most widely used assay for endotoxin, based on the ability of the endotoxin molecule to induce coagulation of proteins in the hemolymph of the horseshoe crab *L. polyphemus*, is notoriously unreliable in biological fluids, because coagulation can

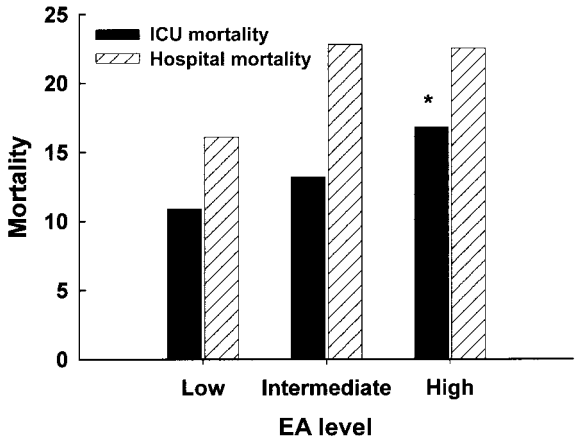

Figure 2. Increasing intensive care unit (ICU) and hospital mortality with increasing levels of endotoxin activity at the time of ICU admission. ICU mortality, $\chi^2 = 4.229$, $P = .04$ (Mantel-Haenzel); hospital mortality, $\chi^2 = 4.343$, $P = .04$ (Mantel-Haenzel).

be induced by other microbial products, such as components of the fungal cell wall, and circulating host-based inhibitors can block the coagulation response to endotoxin [29]. Thus, although a number of cohort studies have suggested that endotoxemia is common [30], at least one found endotoxemia to be inversely associated with gram-negative infection [11], and the unreliability of the limulus-based assay has precluded its use as a diagnostic test.

The chemiluminescent assay uses a specific antibody to the conserved lipid A moiety of the endotoxin molecule to recognize circulating endotoxin on the basis of the ability of the resulting antigen-antibody complex to prime the patient's own neutrophils for an augmented oxidative burst response to a second stimulus. It is thus both sensitive and specific for endotoxin and can be performed in <1 h, which permits the rapid detection of endotoxin in biological samples that include viable neutrophils. However, it uses the patient's own neutrophils as a readout system and so presents inherent limitations; in particular, it is not possible to store specimens for later assaying, and measurements must be performed within 3 h of obtaining the sample. We were unable to obtain reliable results from 9.9% of samples tested, because of discordant results of assay controls (7.1% of all cases), failure of the subject's cells to respond to LPS (0.7% of cases), delay in performing the assay (3.2% of cases), and equipment malfunction (0.7% of cases). Moreover, the assay detects exposed lipid A in the endotoxin molecule and so may not reflect endotoxin bioactivity in vivo.

We have found that endotoxemia is common in a highly heterogeneous population of critically ill patients on the day of their admission to an ICU: more than one-half of all patients have circulating endotoxin levels >2 SDs above those detected in healthy control subjects. Because of variability in the requirement for informed consent to obtain specimens and of differing screening procedures used in the participating study units, this prevalence estimate may not reliably reflect the true prevalence that would be determined from a comprehensive natural history study that recruited all admitted patients. However, despite the high prevalence of endotoxemia, only 4% of the study cohort had gram-negative infections according to the criteria of the CDC. This observed rate of infection may be artefactually low because of concomitant antibiotic use; however the discrepancy between rates of gram-negative infection and endotoxemia suggests that the latter may derive from sources other than invasive gram-negative infection. Although documentation of endotoxemia therefore lacks specificity for a diagnosis of infection, a normal level of circulating endotoxin supports the conclusion that infection is absent: only 1.2% of patients with low levels of endotoxemia had infection with gram-negative organisms, and only 5% of these patients were infected with any organism.

The indigenous flora of the gastrointestinal tract contains large amounts of endotoxin, and translocation of both endotoxin and viable bacteria from the gut has been demonstrated in a variety of animal models, as well as in human illnesses associated with splanchnic hypoperfusion. Consistent with this, we observed that shock was twice as common in patients with increased endotoxin levels, although the observational nature of the present study does not permit a firm conclusion as to whether hypotension was the cause or the consequence of the observed endotoxemia.

However, patients who had intermediate or high levels of endotoxin on the day of admission were clearly a sicker population, as reflected in higher admission APACHE II scores and a greater prevalence of severe sepsis. Moreover, patients with the highest levels of circulating endotoxin had a significantly increased risk of dying while in the ICU. Thus, the presence of endotoxemia identifies a high-risk subpopulation of critically ill patients. Whether this increased mortality risk might be reduced by specific measures to neutralize endotoxin is unknown, but the hypothesis is an attractive one. Not only have the results of animal studies supported a pathogenic role for increased concentrations of endotoxin, but previous analyses of studies designed to neutralize endotoxin in human sepsis have suggested that the greatest potential for benefit occurs in those patients in whom endotoxemia is present, whereas intervention may actually harm those with infection caused by non–gram-negative organisms [31, 32].

Inferences from the present study are limited by the observational nature of the study and the lack of intensive follow-up data that might permit correlations between changes in circulating endotoxin levels and events that reflect clinical improvement or deterioration. Nonetheless, the demonstration of an association between endotoxemia and infection, on the one hand, and an increased risk of adverse outcome, on the other, opens the door to a more rational approach to the management of severe sepsis, guided by the assay of one of its cardinal triggers.

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