Endotoxemia and Bacteremia in Patients With Sepsis Syndrome in the Intensive Care Unit*

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Study objectives: To study circulatory endotoxin (ET) in patients with sepsis syndrome *(SS)* in order to answer three questions: *(a)* How often and at which concentration is ET present in the plasma of patients with SS and is the presence of ET a prognostic marker in this situation? *(b)* Is detection of ET helpful in predicting Gram-negative bacterial infections with or without bacteremia? *(c)* What are the kinetics of clearance of ET concentrations in plasma?

Design: Prospective study of consecutive patients fulfilling Bone's criteria for SS.

Setting: Medical ICU in a teaching hospital.

Patients: The study included 93 patients. The simplified acute physiologic score was 19 ± 6, 49 percent were in shock, and 54 percent were mechanically ventilated. The mortality at day 28 was 53 percent.

Measurements: Endotoxin determinations and blood cultures were performed simultaneously at the onset (day 1) of SS. Samples were collected on several days from 48 patients. Endotoxin concentration was determined using an end point chromogenic Limulus assay. For the first ET determination, the mean circulatory level (mean ± SEM) was calculated among patients with detectable ET, thus excluding patients with a null value for ET.

Results: On day 1, ET was detected in 44 patients (47 percent; 60.2 ± 16.5 pg/ml) and was statistically more frequent in patients with shock, elevated plasma lactate, and organ failure. There was no statistical difference for age, gender, ratio of PaO_2 to fraction of inspired oxygen. Among patients with proven Gram-negative bacterial infection (n = 46), ET was detected in 67 percent as compared with 28 percent without Gram-negative bacterial infection (p = 0.0001). On day 1, among 19 patients who had positive blood cultures with Gram-negative bacteria (GNB), 15 had detectable ET (79 percent, 61 ± 22 pg/ml). In 14 other patients whose blood cultures were positive for GNB but became negative on day 1, 9 had detectable ET (64 percent; 36 ± 6.5 pg/ml). Endotoxin declined linearly between days 1 and 4.

Conclusion: In our study, the plasma ET concentration predicts neither Gram-negative infection, with or without bacteremia, nor the outcome. However, when ET is present in the plasma of patients with SS it remains detectable for a long period of time as compared to its rapid disappearance from plasma of animals or healthy human volunteers receiving ET intravenously. This slow clearance of ET suggests either a continuous release or a defect in its clearance. *(Chest 1994; 106:1194-1201)*

ET=endotoxin; FDA=Food and Drug Administration; GNB=Gram-negative bacteria; LAL=Limulus amebocyte lysate; LPS=lipopolysaccharide; OSF=organ system failure; PRP=platelet-rich plasma; SAPS=simplified acute physiologic score; SS=sepsis syndrome

Key words: endotoxin; Gram-negative bacteremia; sepsis syndrome

Sepsis associated with organ dysfunction or shock, or both, is still a leading cause of death and is on the rise.[1,2] Gram-negative bacteria (GNB) account for approximately 50 percent of documented infections.[3,4] There are several lines of evidence for the participation of endotoxin (ET) originating from GNB in the inflammatory response observed in patients with severe GNB-related sepsis.[5,6] Treatments aimed at neutralizing or antagonizing ET have been tried for more than 20 years. Although initial results with polyclonal[7,8] or monoclonal[9,10] anti-ET antibodies were promising, confirmatory data are lacking. To date, no anti-ET monoclonal antibodies have been approved by the Food and Drug Administration (FDA).[11,12]

The lack of efficacy of the anti-ET drugs could be related in part to the absence of circulatory ET in patients with sepsis syndrome (SS).[13] Surprisingly, few studies have assessed ET level in the plasma of patients with SS, and the results often are contradictory. The observed discrepancies could be explained in several ways: the assays used to measure ET were different, the patients had different types of infections or different levels of severity, the number of ET determinations per patient or the timing of blood cultures differed. Danner et al[14] found ET to be present in 11 of 19 patients with positive GNB blood cultures, but endotoxin also was present in the plasma of 32 patients with septic shock without GNB bacte-

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remia. The sensitivity of the assay for predicting GNB bacteremia was therefore only 58 percent and the specificity, 78 percent.[14] Van Deventer et al[15] used ET as an indicator of the risk of developing SS among high-risk patients. The sensitivity of ET determinations was 79 percent and the specificity, 96 percent. Wortel et al[16] measured plasma ET in a subgroup of 82 patients of the HA-1A (a monoclonal antilipid A antibody) trial and found a sensitivity of 56 percent and a specificity of 82 percent.[16] These studies neither indicated whether the previous blood cultures were positive nor when the first ET assay was performed in relationship to the onset of SS.

We conducted a study to measure circulating ET coupled with blood cultures in patients with a SS as defined by Bone et al[1] in order to answer the following questions: Is ET a prognostic marker of SS and how often and at which concentration is it present in the plasma of patients with SS? Is knowledge of ET concentrations helpful in predicting GNB infection with or without bacteremia? What are the kinetics of ET concentrations in plasma?

Materials and Methods

Patient Eligibility

Over an 18-month period (September 1992 through March 1993), 93 patients admitted to the ICU of Saint Antoine Hospital fulfilled the criteria for SS as defined by Bone et al[1] and were consecutively included in the study. Among these patients, 48 had serial daily samplings over a 10-month period. Shock was defined as a systolic blood pressure below 90 mm Hg for more than 1 h, which was unresponsive to plasma expanders and requiring inotropic or vasoactive agents (dopamine or norepinephrine), or both. Disseminated intravascular coagulation was defined as either a drop in the platelet count of 25 percent or more plus a greater than 20 percent increase in prothrombin or a greater than 20 percent partial thromboplastin time plus D-dimer more than 0.5 $ng \cdot L.^{-1}$ The initial blood samples were obtained as soon as possible after admission and the delay between the beginning of SS (defined by the first organ dysfunction) and the first sampling was noted. The severity was assessed by the simplified acute physiologic score (SAPS)[17] and organ system failure (OSF).[18] Survival was defined as being alive at 28 days after meeting criteria for SS. Immunosuppression was defined as the presence of one of the following: AIDS, solid tumor treated with chemotherapy, any hematologic cancer, or steroid treatment (>0.5 mg/kg of body weight per day). Among the 93 patients who consented to participate, 17 were included in a prospective double-blind, placebo-controlled randomized, multicenter trial of a monoclonal antibody to tumor necrosis factor-alpha. Two patients with purpura fulminans had received HA-1A.

Collection of Data and Blood Sampling

Samples for determination of circulating total ET and for blood cultures were collected simultaneously from each patient. The first sampling was performed after a mean of 16 ± 3 h after the first organ dysfunction.

Endotoxin Assay: Blood (4 ml) was collected through a peripheral vein into nominally sterile ET-free plastic tubes containing sodium heparin, 120 IU (Endotube - Chromogenix). No ET was detected in empty tubes filled with pyrogen-free water. After sampling, the tubes were immediately immersed in ice. Centrifugation was performed within 1 h after sampling (190 *g* for 10 min at 4°C) and platelet-rich plasma (PRP) was separated and stored at −20°C (Falcon tubes) until the assay was performed. The determination of the ET concentration was based on an end point Limulus assay as described by Van Deventer et al.[15] All samples were processed in a laminar flow hood. Inhibiting and enhancing plasma components were eliminated by dilution (tenfold in pyrogen-free water) and heating (15 min at 75°C) before the assay was performed. Diluted heated PRP (50 ml) was incubated in a microtiter plate with the Limulus amebocyte lysate (LAL) (50 ml) for 40 min at 37°C, and chromogenic substrate S 2423 was added followed by 5 min of continued incubation. The optical density values at 405 nm were read after addition of 50 percent acetic acid (50 ml). All assays were performed in duplicate and blanks were obtained by addition of acetic acid prior to addition of the substrate. The ET concentrations were read on a standard curve in PRP. Standard curves were drawn with ET prepared from *Escherichia coli* 0111: B4 diluted in ET-negative PRP from healthy donors. The lower detection limit is 5 pg/ml (0.06 ET unit/ml) and the assay time is 2.5 h. A standard dose of 20 pg/ml of ET (spike) was added to each sample. This procedure enabled us to test the effectiveness of the heating-dilution step. The recovery of spiked ET in PRP from patients is calculated as follows: ([ET] in spiked samples - [ET] in nonspiked samples) × 100/20 and should be between 75 and 125 percent. This methodology has been described and used by Joop et al[19] and is recommended by the FDA guidelines on validation of the LAL test.[20] The within- and between-assay variation coefficients of the assay at 100 pg/ml PRP were 6 and 5 percent, respectively. At 10 pg/ml PRP, values of 10 and 15 percent were obtained.

Blood Cultures: Ten milliliters of blood was distributed into aerobic and anaerobic bottles (BCB Combi set, Roche). According to the results of blood cultures obtained at the time of the first ET determination, GNB infections were classified into three groups: (1) no previous or actual positive blood culture, (2) positive blood cultures 24 to 48 h before ET determination, but no positive blood culture at the time of admission, and (3) blood culture positive at the time of admission.

Statistical Analysis

For continuous variables, means ± SEM were calculated, and absolute and relative frequencies were measured for discrete variables. Differences between groups were examined for statistical significance by a Mann Whitney U Test in the case of continuous variables and by Fisher's exact test in the case of discrete variables.

For the first ET determination, the mean circulatory level was calculated among patients with detectable ET, thus excluding patients with a null value for ET. For the sequential analysis of ET, the means were calculated including all patients with detectable ET at least once. Most of these patients with initial positive circulatory ET tested negative on a secondary test, so these null ET values were integrated into the calculations of means of ET at days 2, 3, and 4.

Results

General Characteristics of the Patients With Sepsis Syndrome

Patients: The severity of the condition of the 93 patients studied was assessed by a SAPS of 19 ± 6 (Table 1). The condition of the patients at the time of the first sampling was as follows: shock, 49 percent; mechanical ventilation, 54 percent; anuria requiring hemodialysis, 6 percent. The population was mainly

Table 1—*Characteristics of the Global Population With Sepsis Syndrome**

Characteristics	All Patients (n=93)	Survivors (n=44)	Nonsurvivors (n=49)	Probability Value
Age, yr	52.7±2	53.6±3	51.9±2.6	>0.1
Males/Females	55/38	27/17	28/21	>0.1
Body temperature				
Hyperthermia, >38.3°C	77 (83)	40 (91)	37 (76)	0.05
Hypothermia, <35.6°C	16 (17)	4 (9)	12 (24)	
Presence of shock	46 (49)	16 (35)	30 (65)	0.02
Arterial lactates levels, mmol/L	5.4±0.5	4.3±0.6	6.3±0.8	0.06
Arterial pH	7.37±0.01	7.41±0.01	7.34±0.02	0.003
Disseminated intravascular coagulation	50 (54)	22 (44)	29 (56)	>0.1
PaO_2/FIo_2 ratio†	287±14	320±18	258±20	0.03
SAPS	19±0.7	16.5±0.8	21.3±1	0.0003
OSF	1.7±0.13	1.07±0.14	2.27±0.18	0.0001
GNB infection	46 (49)	24 (55)	22 (45)	>0.1
Site of infection				0.003
Lung	25 (27)	8 (18)	17 (35)	
Kidney	16 (17)	13 (30)	3 (6)	
Peritoneum	13 (14)	8 (18)	5 (10)	
Cerebrospinal fluid	5 (5)	4 (9)	1 (2)	
Miscellaneous	34 (37)	11 (25)	23 (47)	

*Quantitative values are expressed as the mean ± SEM and comparisons were performed with a Mann-Whitney U test. Qualitative values are expressed as numbers and a percentage within each group and statistical comparisons were performed with Fisher's exact test.
†FIo_2=fraction of inspired oxygen.

patients in the medical ICU with immunosuppression in 49 percent. Chemotherapy-induced leukopenia (<500 leukocytes/mm^3) was observed in 9 patients. Mortality was 53 percent.

Documentation of Infection: Sepsis syndrome was related to a GNB infection in 49.4 percent, to another documented infection (mainly Gram-positive cocci) in 33.3 percent, and to noninfectious etiology (even after autopsy) in 17.2 percent of the patients. The primary sites of infection were the lung (27 percent), the kidney (17 percent), the peritoneum (14 percent), the meninx (5 percent), and miscellaneous areas (37 percent [central venous line, endocarditis, mediastinitis, gynecologic or pharyngeal infection]).

Table 2—*Results of Initial Plasma Endotoxin Determination According to the Etiology of the Sepsis Syndrome*

Etiology of SS	ET >5 pg/ml, No. (Mean±SEM)	ET <5 pg/ml, No.	Probability Value
Global population, n=93	44 (60.2±16.5)	49	
Documented infection, n=77	37 (52.5±13.5)	40	NS*
No infection, n=16	7 (101±79)	9	
GNB infection, n=46	31 (59.8±15.7)	15	0.0001
No GNB infection, n=47	13 (61.2±43)	34	
GNB bacteremia, n=33	24 (51.7±14)	9	0.0003
Gram-positive cocci bacteremia, n=19	2 (13±3)	17	

*NS, not significant.

Results of Initial Endotoxin Determination

Endotoxin was detectable in the plasma of 44 patients (47 percent). Among these patients, the mean value of circulatory ET was 60.2 ± 16.5 pg/ml (range, 6 to 574 pg/ml). It is interesting to note that patients without a documented source of infection had detectable ET at a frequency similar to the rest of the population with quantitative values even higher, although the difference was not statistically significant (Table 2). The patients with GNB infections were more frequently endotoxemic than pa-

Table 3—*Characteristics of the Patients With or Without Detectable Circulatory Endotoxin*

Characteristics	ET >5 pg/ml, No. (Mean±SEM)	ET <5 pg/ml, No. (Mean±SEM)	Probability Value
Population, n=93	44	49	
Age, yr	50.4±2.7	54.8±2.8	>0.1
Shock +, n=46	29 (70.7±22.8)	17	0.002
Shock −, n=47	15 (40±20)	32	
Lactates levels, mmol/L	6.8±0.85	3.9±0.5	0.02
PaO_2/FIo_2 ratio, mm Hg*	285±16	290±25	>0.1
SAPS	20.8±1.1	17.5±0.8	0.1
OSF	2.1±0.2	1.3±0.1	<0.001
Survivors, n=44	17 (55±22)	27	0.1
Nonsurvivors, n=49	27 (63.5±23)	22	

*FIo_2=fraction of inspired oxygen.

Table 4—*Characteristics of Patients With a Documented Gram-negative Bacterial Infection With or Without Detectable Circulatory Endotoxin*

Characteristics	ET >5 pg/ml, No. (Mean ± SEM)	ET <5 pg/ml, No. (Mean ± SEM)	Probability Value
Population, n=46	31	15	
Age, yr	49.8 ± 3.2	65 ± 3.8	0.007
Shock +, n=26	20 (65.6 ± 19.5)	6	0.1
Shock −, n=20	11 (49.3 ± 27.7)	9	
Lactates levels, mmol/L	6.2 ± 0.9	2.9 ± 0.8	<0.05
PaO_2/FIo_2, mm Hg*	285 ± 29	307 ± 28	>0.1
SAPS	19 ± 1.2	16.7 ± 1.4	>0.1
OSF	1.9 ± 0.2	1.2 ± 0.3	<0.05
Survivors, n=24	15 (61.4 ± 24.8)	9	>0.1
Nonsurvivors, n=22	16 (58.3 ± 20.6)	6	

*FIo_2=fraction of inspired oxygen.

tients without GNB infection, but once ET was present, the quantitative values were similar in the two groups (Table 2). According to the primary site of infection, ET was detected in 40, 50, 54, and 80 percent, respectively, for lung, kidney, peritoneum, and cerebrospinal infections.

Endotoxin was detected significantly more often in patients with shock, multiple organ dysfunctions, or elevated plasma lactate levels. Age, gender, SAPS, or outcome were not significantly different among patients with or without detectable plasma ET (Table 3).

The results for patients with proven GNB infection are indicated in Table 4. The endotoxemic patients were younger and had a tendency to be more severely ill than the nonendotoxemic patients. Twenty-six patients had shock with ET detectable in 77 percent (66 ± 6 pg/ml), and the frequency of detectable endotoxemia was similar whether they had bacteremia (n = 17; ET, 74 percent) or not (n = 9; ET, 67 percent). The patients with GNB bacteremia were studied in terms of the delay between the blood culture and the first ET determination. Among the 19 patients with positive GNB blood cultures simultaneous to the first ET determination, ET was detected in 15 (79 percent, 61 ± 22 pg/ml). The four patients without endotoxemia had *E coli* (n = 2), *Haemophilus influenzae* (n = 1), and *Bacteroides fragilis* (n = 1) bacteremia. Fourteen other patients had positive blood cultures less than 48 h before the first sampling for ET, while the blood cultures performed simultaneous to the ET determination were negative (they had received antibiotics in the meanwhile). In this subgroup, ET was detected in 9 patients (64 percent; 36 ± 6.5 pg/ml), while 5 patients with *E coli* bacteremia had no detectable ET. The prevalence of detectable ET or its concentration was not statistically different between the patients with previous or simultaneous positive GNB blood cultures. Thirteen patients had a documented GNB infection without any positive blood culture. In this group, ET was detected in 7 patients (54 percent; 87.6 ± 52.6). Among the six patients without endotoxemia, three had peritonitis and three, pyelonephritis. At last, there was no difference in the detection or concentrations of ET between GNB-infected patients with (24 of 33; 51.7 ± 15 pg/ml) or without bacteremia (7 of 13; 87.6 ± 52.6 pg/ml).

Serial Determinations of Endotoxin

Measurements of ET were performed on several days in 48 patients until resolution of the SS or death. Endotoxin was recovered at least once in 31 patients. In only 2 cases was ET not detected in the first sample on day 1 but on day 3 for a patient with shock and a severe *Staphylococcus aureus* infection and on day 4 for a second patient with pyelonephrosis due to *Klebsiella pneumoniae*. For both cases, the ET values were just above the detection limit of 5 pg/ml. The percentage of patients with detectable circulating ET declined with time with 94 percent on day 1, 78 percent on day 2, 40 percent on day 3, and 27 percent on day 4 ($p < 0.01$ by one-way analysis of variance). As indicated in Figure 1, the mean values of ET decreased progressively with time. Seventeen patients with GNB bacteremia had serial determinations of ET. All patients had an adequate antibiotic regimen, and blood cultures were negative on day 2 for all patients. Endotoxin increased from day 1 to day 2 in 5 patients, while ET constantly declined in the 12 other patients. There was no statistical difference in mortality among these two groups of patients.

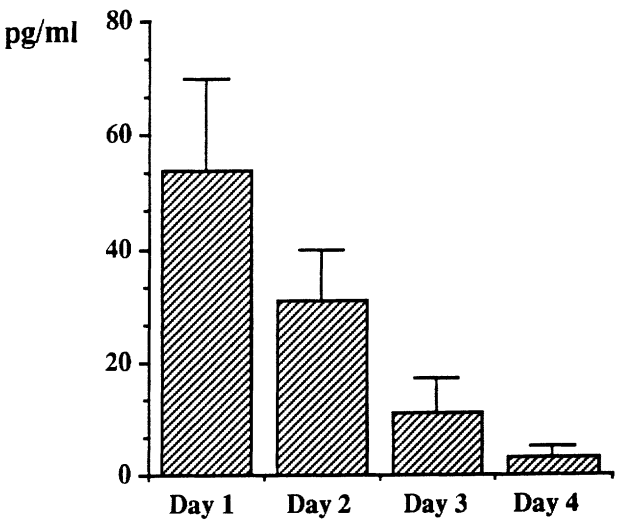

FIGURE 1. Serial determinations of ET level in the plasma. The results are expressed as mean ± SEM for all patients.

Discussion

Contribution of Endotoxin Determinations to the Selection of High-Risk Patients

Sepsis and septic shock remain a leading cause of death, and there are several lines of experimental evidence to suggest that ET is implicated. The natural history of ET in human septic disease is not well documented, although extensive antilipopolysaccharide (LPS) treatment trials have been performed. In this study, we investigate whether a rapid endotoxin assay would be useful in identifying a population with a high risk of death and who could benefit from anti-LPS or anticytokine therapy. We found that quantitative or qualitative values for ET did not discriminate between patients with or without documented infection. Moreover, we were unable to find any differences among patients with GNB infection whether they had bacteremia or not and this further questions the subgroup analysis performed in the recently published anti-LPS treatment trails.[9-11] The lack of differences in the level of ET among survivors and nonsurvivors also has been found by Casey et al[21] and the relationship between plasma ET levels and severity of the condition has been questioned.[22] However, Danner et al[14] found a higher mortality among endotoxemic patients, and in the case of systemic meningococcal disease, there was also a correlation between endotoxemia and the mortality rate[23] as well as between ET level as assessed by LAL assay and by gas chromatography.[24] These differences could be partly explained by a variation in the degree of ET toxicity depending on the strains. For example, Danner et al[14] showed that *Psuedomonas aeruginosa*-infected dogs were sicker than dogs infected with *E coli* despite an ET concentration tenfold lower.[25]

We found that simple clinical and biological criteria such as the presence of shock, acidosis, hypoxemia, hypothermia, and the site of infection were good predictors of mortality. Calandra et al[26] found similar results in a multicriteria analysis, and in this study tumor necrosis factor was not a good factor for predicting mortality. Casey et al[21] were required to express LPS and cytokines values as a score in order to find a correlation between this score and mortality. Even with this score, the prediction was weak for the patients with sepsis of intermediate severity.

In summary, we believe that initial circulatory ET assessed by the LAL chromogenic method should not be used as a criteria for inclusion in anticytokine trials or even in anti-LPS trials.

Prediction of Gram-Negative Bacteremia With Endotoxin

It is difficult to predict GNB infection from simple clinical factors.[27] The accuracy of prediction by physicians of GNB bacteremia is no more than 40 percent either in the United States[9] or in France.[28] So ET determinations could be useful for that purpose. This has been tried since 1970[29] with overall poor results. In 1979, Elin[30] analyzed the results of 17 reported studies and concluded that the clinical relevance of the LAL assay for predicting GNB bacteremia could not be established. The breakthrough came with the development of an increased sensitivity of the LAL assay by using chromogenic substrates and with the improvement of methods for eliminating plasma activators and inhibitors.[31] However, even with these new assays, the recently published results of ET are mixed (Table 5). These discrepancies could be explained by several factors: the assays for ET are not standardized (gelification or chromogenic methods, different techniques for elimination of plasma activators and inhibitors, different LPS and vehicles used for standard curves). The se-

Table 5—*Prediction of Gram-negative Bacteremia With the Limulus Amebocyte Lysate Assay in Patients With Sepsis in Various Studies*

Study	Year	Inclusion Criteria	Assays for ET	Detection Limit, pg/ml	Patients	Sensitivity, %	Specificity, %
Thomas et al[32]	1984	Risk of GNB bacteremia	Chromogenic	10	51	67	97
Pearson et al[33]	1985	Septic patient ?	Gelification, chromogenic	?	45	90	94
Van Deventer et al[15]	1988	Risk of GNB bacteremia	Chromogenic	5	473	79	96
						84*	60*
Shenep et al[34]	1988	Risk of GNB bacteremia	Gelification	25	36	90	81
Brandtzaeg et al[23]	1989	Meningococcal infection	Chromogenic	25	42	69	100
Danner et al[14]†	1991	Septic shock	Chromogenic	10	100	58	78
Wortel et al[16]	1992	SS	Chromogenic	3	82	56	82
Present study	1993	SS	Chromogenic	5	93	79	61
						73‡	67‡

*After combination of data gathered from patients with an initial SS and patients with SS during the follow-up.
†Endotoxin measurements were made every 4 h during the first 24 h and simultaneous blood cultures were not always performed.
‡Results obtained for patients with previous or simultaneous GNB bacteremia.

verity of the patients' conditions varies from one study to another, with several even including patients without sepsis syndrome.[15] The reference to simultaneous blood cultures is sometimes lacking.[14] The strains responsible for sepsis may have different releasing ET characteristics (*ie*, in the study of Brandtzaeg et al[23] *Neisseria meningitidis*-related infection was only explored). Previous GNB bacteremia could interfere with the calculated sensitivity and specificity of ET determinations and should be indicated (*ie*, this information is lacking in the study by Wortel et al[16]).

In our patients, the sensitivity and specificity of ET for GNB bacteremia at the time of admission were 79 percent and 61 percent, respectively (Table 5). When all patients with GNB bacteremia were pooled (n = 33), the sensitivity was 73 percent and specificity, 67 percent.

What are the hypotheses to explain the lack of ET plasma detection while GNB are present in the blood (false-negative results)?

1. Presence of plasma inhibitors of the LAL. This has been ruled out, since exogenously added ET (spiked sample) was recovered, indicating that the dilution-heating procedure was accurate.

2. Endotoxin with no activity on the LAL. This biologically inactive ET already has been described.[35] This outlines that the LAL assay tests the capacity of plasma to activate the LAL cascade, but that it is not a true and direct measurement of ET.

What are the hypotheses to explain ET plasma detection in spite of the absence of documented GNB infection (false-positive results)?

1. Endotoxin translocating from the digestive tract. Intestinal endotoxemia without bacteremia has been described in several experimental animal models.[36] Acute bowel ischemia is a common phenomenon in patients with septic shock and could explain the low level of ET in patients without GNB infection. Interestingly, among the 47 patients without GNB infection, ET was detected in 45 percent when there was shock as compared to 15 percent in patients without shock ($p < 0.05$). Furthermore, liver failure or cirrhosis may impair the ET hepatic clearance.[37-38] The contribution of the liver cannot be assessed in our study because of the small size of the population.

2. Yeast cell wall component activation of the LAL. A specific assay has been developed in order to render the assay completely ET-specific.[39] We did not use this assay, but as in the study of Wortel et al,[16] two patients in our study with pure fungal sepsis had no positive LAL results.

3. Low number of circulating bacteria. It is now recognized that ET is normally produced during the normal growth of GNB so we can speculate that at the beginning of a GNB infection, a small burden of GNB can produce ET while the standard microbiological methods are not sensitive enough to detect the bacteria. Howver, in the few studies with quantitative blood cultures, there was no clear correlation between the level of ET and bacterial density.[23]

Determination of Clearance of ET

Among patients with detectable ET and with serial sampling (31 of 48), ET was positive in the first sample in 29 of 31 of the cases. This result suggests that the repetition of ET measurements more than 24 h after the first sampling does not strongly increase the detection of endotoxemia, but the repetition of the samples, every 4 h during the first 24 h, enabled Danner et al[14] to detect ET after the first sample in more than 50 percent of the patients. In the study of Wortel et al,[16] only 17 percent of the initially nonendotoxemic placebo recipients became endotoxemic within 24 h. Our high rate of detection of ET in the first sample could be explained by the short delay between the beginning of the SS and the first sampling for ET.

The mechanism of ET clearance is still not completely understood but involves natural anti-ET antibodies, binding to high-density liproproteins and macrophages. Endotoxin administered by bolus infusion in animals is cleared in less than 3 h, while in slow infusion it is more slowly.[38] In a recent report, a laboratory worker self administered intravenously 1 mg of *Salmonella minnesota* ET, a dose that is about 4,000 times higher than the dose used for studying ET effects in healthy volunteers. He developed shock and multisystem organ failure. The circulatory cytokines (tumor necrosis factor alpha, IL-1, IL-6, and IL-8) were very high 3.6 and 6.8 h after the injection, but ET in serum was only slightly elevated 6.8 h after injection and below the detection limit after 11.5 h.[40] In animal models, lethal experimental *E coli* infection may be associated with transient endotoxemia[41] or persistent endotoxemia even though *E coli* is no longer detected in blood.[42] In human sepsis, ET usually is present for a longer period. Among the initially endotoxemic patients, ET is still detectable in 25 percent[14] to 62 percent[16] after 24 h. In our patients with GNB infections, 64 percent were endotoxemic after day 1, while at that time none were still bacteremic. Brandtzaeg et al[23] found that ET was always negative within the first 40 h and never found an increase of ET. McCartney et al[43] found that all 9 patients with secondary negative ET survived, while all of the 18 patients with persistent ET ultimately died. In a few patients with GNB sepsis, Shenep et al[34] documented a secondary increase of free ET induced by antibiotics. This potential ET-releasing effect of antibiotics has been reviewed recently[41] and its relevance to human pa-

thology seems questionable. Among the 17 GNB bacteremic patients of our study with serial ET determinations, we were unable to find any relationship between evolution of ET level and outcome. However, the design of our study does not allow us to draw firm conclusions about the effect of antibiotics on ET release.

Conclusion

In our study, plasma ET concentrations predict neither Gram-negative infection with or without bacteremia, nor the outcome. However, when ET is present in the plasma of patients with SS, it remains detectable for a long period of time as compared with its rapid plasma disappearance in animals or healthy human volunteers receiving ET intravenously. This slow clearance of ET suggests either a continuous release or a defect in its clearance.

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