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Endotoxaemia in patients with severe sepsis or septic shock

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Abstract *Objective*: To examine the incidence and the bacteriological and clinical significance of endotoxaemia in ICU patients with severe sepsis or septic shock.
Design: Prospective review.
Setting: A 15-bed general ICU in a university hospital.
Patients: One hundred sixteen patients hospitalised in our ICU fulfilling Bone's criteria for severe sepsis or septic shock and with an available early endotoxin assay (chromogenic limulus assay).
Interventions: None.
Measurements and results: The clinical characteristics of the population were: age 63.6 ± 11.4 years; SAPS II: 45.4 ± 15.6; mechanical ventilation: 72.4 %; septic shock: 51.7 % (n = 60); bacteraemia: 28.4 % (n = 33); gram-negative bacteria (GNB) infection 47.4 % (n = 55); ICU mortality: 39.6 % (n = 46). Detectable endotoxin occurred in 61 patients (51.2 %; mean level: 310 ± 810 pg/ml). There was no relationship between detectable endotoxin and severity of infection at the moment of the assay. Endotoxaemia was associated with a higher incidence of bacteraemia (39.3 % vs 16.3 %; p = 0.01). There was a trend (p = 0.09) towards an association between positive endotoxin and gram-negative bacteraemia or GNB infection but this was non-significant. This relationship became significant only in the case of bacteraemia associated with GNB infection irrespective of the site of infection.
Conclusion: Early detection of endotoxaemia appeared to be associated with GNB infection only in cases of bacteraemic GNB infection. Early endotoxaemia correlated neither to occurrence of organ dysfunction nor mortality in patients with severe sepsis or septic shock. This study suggests that the use of endotoxaemia as a diagnostic or a prognostic marker in daily practice remains difficult.

Key words Endotoxaemia · Severe sepsis · Septic shock · Clinical study · Outcome · Bacteraemia · Gram-negative bacteria infection

Introduction

Sepsis associated with shock and/or organ dysfunction is still a leading cause of admission to an intensive care unit (ICU) and of death [1]. Gram-negative bacteria (GNB) infections have aroused particular interest for many years due to their high incidence and endotoxin. The endotoxin is a cell wall component exclusively found in gram-negative bacteria (GNB). Endotoxin was thought to be one of the most important toxins involved in the development of septic shock and multiple organ failure associated with gram-negative bacteria infections [2]. However, results from animal models of septic shock have questioned the role of endotoxin in pathogenesis and cardiovascular abnormality [3, 4, 5]. In the same way, its role in human infections remains

controversial. Firstly, many clinical trials using anti-endotoxin therapy have yielded inconclusive results [6]. However, these data are challenged again because of the endotoxin binding specificity of the main monoclonal antibodies used (HA-1A) [7] and also because of the large number of patients with gram-positive infections enrolled. Secondly, in clinical practice, except in the context of *Neisseria meningitis*-related infections in which endotoxaemia was constantly detected and had a high prognostic value in the case of purpura fulminans [8], there was no proven evidence of the correlation between endotoxin (detection and levels) and the clinical outcome [9]. Thirdly, few studies have assessed the prognostic value of the detection of circulatory endotoxin in in the clinical practice of ICUs [10, 11, 12]. Finally, these studies included a lot of immunocompromised patients [10, 11].

The aim of this study was to evaluate the interest of an early endotoxin assay in the current clinical practice of ICUs. We evaluated the clinical prognostic and bacteriological values of the detection of circulatory endotoxin in in the early phase of severe sepsis in a general population of patients admitted to our ICU. The relationship between the detection of endotoxaemia, the occurrence of organ failure and fatal outcome was examined.

Patients and methods

Patient eligibility

This prospective review included ICU patients who had Bone's criteria for severe sepsis [13] and an available circulatory endotoxin assay during the first 24 h of infection. Infection could have been the reason for ICU admission or it appeared during the ICU stay. The diagnosis of severe sepsis was based on Bone's criteria [13] according to the following definitions:

- Severe sepsis is a systemic response to apparent clinical infection with fever or hypothermia (temperature > 38.3 °C or < 36.5 °C), tachycardia (heart rate higher than 90 beats/min in the absence of adrenergic receptor ß-blockade) and tachypnoea (respiratory rate of > 20 breaths/min or the requirement of mechanical ventilation) associated with two of the following five signs of systemic toxicity or peripheral hypoperfusion: an increased plasma lactate concentration above 2 mmol/l; arterial hypoxaemia (partial pressure of oxygen (PaO_2) < 75 torr [< 10 kPa] or a PaO_2/FIO_2 ratio < 250); acute renal failure (urinary output < 0.5 ml/kg of body weight/h); sudden decrease of mental acuity; reduction of the platelet count to less than half of the baseline value or below 100,000 platelets/mm^3.
- Septic shock is a severe sepsis with hypotension with systolic blood pressure lower than 90 mmHg or a reduction of more than 40 mmHg from baseline in the absence of antihypertensive agents despite adequate fluid resuscitation or the use of vasopressor agents to maintain blood pressure.

For the endotoxin assay, blood samples were collected in sterile, endotoxin-free heparinised tubes. They were centrifuged for immediate separation and plasma was stored at –70 °C for assay. Before being assayed, the samples were diluted by 1:5 or 1:10 and inactivation of inhibitors was obtained by heating the sample for 15–20 min at 75 °C. A chromogenic kinetic limulus amebocyte lysate assay (LAL assay: Bio Whittaker KQCL 1000, Bio Whittaker France, 94120 Fontenay sous Bois) was used to measure the endotoxin. A result 0.1 EU/ml (equivalent to 10 pg/ml) or more was considered as positive. Even if daily serial assays were performed, only the first result was analysed in this study. Attribution of infection by the physicians was blinded to the endotoxin data and based only on Bone's criteria. In accordance with French law, no informed consent was necessary, given that this study did not modify the existing diagnosis or the therapeutic strategy.

To analyse the clinical prognostic value of the detection of a circulatory endotoxin, clinical and biological data were collected on the day of the endotoxin assay. The biological data included: arterial blood gas determinations, blood and urine electrolyte values and creatinine level, arterial lactate concentration, blood cell counts, C-reactive protein, Quick time, partial thromboplastin, fibrinogen and D-dimer. Definitions of organ failures were the following:

- Acute respiratory distress syndrome (ARDS): association of PaO_2/FIO_2 ratio below 200 mmHg regardless of positive end-expiratory pressure (PEEP) level, bilateral parenchyma infiltrates demonstrated on frontal chest radiograph and pulmonary wedge pressure less than 18 mmHg when measured or no clinical evidence of left atrial hypertension [14].
- Disseminated intravascular coagulation (DIC): either a drop in the platelet count of 25 % or more plus a decrease greater than 20 % in Quick time or an increase greater than 20 % in partial thromboplastin time plus D-dimer more than 0.5 ng/l [11].
- Acute renal failure: acute anuria or need for dialysis or serum creatinine of 180 μmol/l or more [10].

The severity of the disease was assessed by the simplified acute physiologic score (SAPS II) [15]. The analysed mortality was ICU mortality.

To examine the bacteriological prognostic value of the detection of a circulatory endotoxin, the bacteriological results were taken into account during the 48 h of the endotoxin assay and included: blood and urine cultures, pulmonary samples (bronchoalveolar lavage or tracheal protected secretion), appropriate (surgical or not) local samples. GNB infection was defined as follows: presence on bacteriological samples of a GNB organism and infection considered by the physicians in charge of the patients to be related to these bacteriological results. It could be a monomicrobial or polymicrobial infection.

In the case of the presence of one of the following criteria: AIDS, solid tumour treated with chemotherapy, haematological cancer, steroid treatment (> 0.5 mg/kg of body weight per day), leukopenia less than 1000 leucocytes/mm^3, the patients were considered as immunocompromised [11].

Statistical analysis

Two studies have been conducted on a global population: a survival/mortality study and an analysis according to the absence or the presence of endotoxaemia. For continuous values, means ± SD were calculated. Comparisons between groups were made using a Mann-Whitney test for continuous values and two-tailed chi square (or Fisher's exact test, as appropriate) in the case of discrete variables. A p value less than 0.05 was considered as significant.

Table 1 Characteristics of the global population and results of the survival/mortality study[a] (FIO_2 fraction of inspired oxygen, *SAPS II* Simplified Acute Physiologic Score, *ARDS* acute respiratory distress syndrome, *DIC* disseminated intravascular coagulation, *GNB* gram-negative bacteria)

Characteristics *n*	All Patients 116	Survivors 70	Nonsurvivors 46	*p*
Endotoxemia (%)	61 (51.2)	36 (51.4)	25 (54.3)	0.9
Clinical data				
Age, year	63.6 ± 11.4	61.5 ± 15.6	66.9 ± 12	0.06
Sex (M/F)	78/38	48/22	30/16	0.8
Ventilatory support required (%)	84 (72.4)	50 (71.4)	34 (73.9)	0.9
SAPS II	45.4 ± 15.6	39.9 ± 12.3	53.9 ± 16.5	< 0.0001
Septic Shock (%)	60 (51.7)	29 (41.4)	31 (67.3)	0.01
ARDS (%)	25 (21.5)	8 (11.4)	17 (37)	0.002
Renal failure (%)	51 (44)	24 (34.3)	27 (58.7)	0.01
DIC (%)	25 (21.5)	10 (14.2)	15 (32.6)	0.03
Biological data				
PaO_2 (mm Hg)/FIO_2 ratio	169 ± 89.6	194.9 ± 84.9	128.9 ± 82.9	0.002
Arterial lactate levels (mmol/L)	3.78 ± 3.21	2.9 ± 1.26	5.1 ± 4.54	0.001
Arterial pH	7.36 ± 0.14	7.38 ± 0.09	7.32 ± 0.12	0.005
C Reactive Protein (mg/L)	166.1 ± 116.9	176.5 ± 105.3	148.6 ± 131.3	0.09
Leucocyte (× 109/L)	13.7 ± 8.1	14.8 ± 8.3	11.8 ± 7.3	0.05
Creatinine (μmol/L)	123 ± 48.5	117.2 ± 77.4	132.2 ± 80	0.16
Bacteriological data				
Respiratory infection (%)	57 (49.1)	35 (50)	22 (47.8)	0.8
Abdominal infection (%)	38 (32.8)	24 (34.3)	14 (30.4)	0.9
Other site of infection (%)	11 (9.5)	5 (7.1)	6 (13)	0.6
Undetermined site of infection (%)	10 (8.6)	5 (7.1)	5 (10.9)	0.9
Positive blood culture (%)	33 (28.4)	19 (27.1)	14 (30.4)	0.9
GNB bacteremia (%)	23 (19.9)	13 (18.6)	10 (21.7)	0.8
GNB on local samples (%)	52 (44.8)	31 (44.3)	21 (45.6)	0.9

[a] Quantitative values are expressed as the mean ± standard derivation and comparisons were performed with a Mann-Whitney test. Statistical comparisons of qualitative values were performed with two-tailed chi square or Fisher's exact test, as appropriate

Results

General characteristics

Over a 4-year period (February 1992 to May 1996), this study included 116 patients. A circulatory endotoxin was detectable in 61 patients (51.2 %), with a mean value of 310 ± 810 pg/ml. Endotoxin assays were obtained at admission from 92 patients (81 %) and, because of sepsis during ICU hospitalisation, from 24. The main clinical characteristics of the population studied are shown in Table 1. Immunodepression occurred in 4.3 % of patients and concerned five patients treated with steroids. The primary sites of infection were the lungs (48.3 %). The infection was documented in 68.9 % of the patients and was related to a GNB in 47.4 % (55/116). Blood cultures were positive in 28.3 % of the patients. The ICU mortality rate was 39.6 %.

In univariate analysis, the presence of a detectable circulatory endotoxin was not statistically different in the survivors and the non-survivors (Table 1). Mortality was statistically correlated with organ failures (shock, ARDS, renal failure, DIC), biological sign of dysoxia (PaO_2/FIO_2 ratio, arterial lactate level and pH) and severity of disease according to the SAPS II score.

Early circulatory endotoxin detection was not correlated with the severity of the infection. There was no statistical difference in SAPS II, PaO_2/FIO_2 ratio, pH, arterial lactate levels, organ dysfunction, presence of septic shock and the mortality between patients with or without detectable endotoxins (Table 2). The higher requirement for mechanical ventilation in the non-endotoxin patients was related to the higher incidence of respiratory infection in this group of patients.

In the non-endotoxin group we found a gram-positive bacteria (GPB) or GNB related infection in 25.5 % (14/55) and 38.2 % (21/55) of the patients, respectively. The same incidences in the endotoxin group were 16.4 % (10/61) and 55.7 % (34/61), respectively. These results, like the incidence of non-documented infection (32.7 % (18/55) in non-endotoxin patients and 27.9 % (17/61) in the endotoxin group) were not different ($p = 0.09$ for GNB infection). The circulatory endotoxin detection was associated with a higher incidence of bacteraemia (39.3 % vs 16.3 %, $p = 0.01$), but not particularly with gram-negative bacteraemia. Among the bacteraemic patients, the detected organism in blood cultures

Table 2 Characteristics of the patients with or without detectable circulatory endotoxin[a] (*FIO_2* fraction of inspired oxygen, *SAPS II* Simplified Acute Physiologic Score, *ARDS* acute respiratory distress syndrome, *DIC* disseminated intravascular coagulation, *ET* endotoxin, *MOF* multiple organ failure)

Characteristics *n*	ET < 0.1 Ul/ml 55	ET ≥ 0.1 Ul/ml 61	*p*
Clinical data			
Age, year	63 ± 15.7	64.2 ± 13.4	0.9
Sex (M/F)	35/20	43/18	0.5
SAPS II	47 ± 16.1	43.6 ± 15.1	0.3
Ventilatory support required (%)	46 (83.6)	38 (62.3)	0.01
Septic Shock (%)	27 (49.1)	33 (54.1)	0.7
ARDS (%)	15 (27.2)	10 (16.3)	0.2
Renal failure (%)	25 (45.5)	26 (42.6)	0.9
DIC (%)	12 (21.8)	13 (21.3)	0.9
Death (%)	21 (38.1)	25 (40.9)	0.9
Death during the first 24 hours (%)	5 (8.2)	10 (18.2)	0.37
Death with MOF after the first day (%)	10 (16.4)	8 (14.5)	0.6
Biological data			
PaO_2 (mm Hg)/FIO_2 ratio	162.3 ± 86.9	177.4 ± 93.4	0.5
Arterial lactates levels (mmol/L)	3.58 ± 1.9	3.96 ± 4.0	0.3
Arterial pH	7.36 ± 0.1	7.35 ± 0.13	0.9
C Reactive Protein (mg/L)	170.3 ± 117	162.1 ± 117.8	0.7
Leucocyte (× 109/L)	14.7 ± 7.9	12.8 ± 8.3	0.1
Serum creatinine (μmol/L)	124 ± 74.9	122 ± 82.3	0.4

[a] Quantitative values are expressed as the mean ± standard derivation and comparisons were performed with a Mann-Whitney test. Statistical comparisons of qualitative values were performed with two-tailed chi square or Fisher's exact test as appropriate

was GNB for 79.2 % of bacteraemia in the endotoxin positive group and 44.4 % without endotoxin detection ($p = 0.09$).

Even if no significant correlation appeared between GNB infection and endotoxaemia detection, the site of sepsis and incidence of bacteraemia interacted in this result. Indeed, 22 GNB infections were secondary to pulmonary sepsis and 26 to abdominal or urinary sepsis. Endotoxin detection seemed to be more frequently associated with abdominal or urinary GNB infections (73 %, 19/26) than GNB pulmonary infections (45.5 %, 10/22) ($p = 0.09$). Another confounding factor in these results was the incidence of positive bacteraemia. The incidence of positive bacteraemia, according to the infection site and irrespective of bacteria-related sepsis, was 10.5 % (2/57) for pulmonary and 36.8 % (14/38) for abdominal or urinary infections ($p < 0.001$).

For GNB pulmonary infection without positive bacteraemia, the incidence of endotoxin detection was 42 % (8/19), but it rose to 66 % (2/3) in the case of bacteraemia associated to GNB pulmonary sepsis. In the same way, the incidence of positive endotoxaemia in the case of GNB abdominal or urinary sepsis was 50 % (6/12) without associated bacteraemia ($p = 0.7$ compared to GNB pulmonary infection without bacteraemia), but 92.9 % (13/14) in the case of GNB bacteraemic abdominal or urinary infection ($p = 0.3$ compared to GNB pulmonary infection with bacteraemia). Irrespective of the sepsis site, an endotoxin was detected in 45.2 % (14/31) of non-bacteraemic GNB infections (with detection incidence not different at pulmonary, abdominal or urinary sites) and in 88.2 % (15/17) of bacteraemic GNB infections ($p = 0.005$). In the seven other GNB infections (3 meningitides, 2 catheter-related infections, one mediastinal infection and one undetermined) we found the incidence of GNB bacteria equal to 85.7 % (6/7) and the incidence of positive endotoxaemia 71.4 % (5/7). Endotoxin detection to predict GNB infection therefore depended on the bacteraemia associated with sepsis.

Discussion

In this study, an early endotoxin assay appeared not to be a useful predictor of clinical severity and of outcome among patients with severe sepsis admitted to the ICU. Like some previous studies conducted in ICUs [10, 11] and a recent meta-analysis including 738 patients with suspected sepsis [9], we observed no relation between positive endotoxaemia and mortality. In two studies [9, 10], positive endotoxaemia has been found to be associated with a higher mortality only in patients with septic shock and proven bacteraemia. The present study did not find any relationship between positive endotoxaemia and severity of sepsis. These results differ from Danner and Guidet's studies [10, 11] (Table 3). For these authors [10, 11], positive endotoxaemia was associated with severe manifestations of sepsis syndrome, including cardiac depression, lower systemic vascular

Table 3 Early endotoxemia impact in ICU patients with severe sepsis. Comparisons of three studies (*ET* endotoxin, *GNB* gram-negative bacteria, *Na* not available)

n	Danner et al. [10] 100	Guidet et al. [11] 93	present study 116
Characteristics of study			
type	prospective	prospective	prospective
endotoxin assay	chromogenic	chromogenic	chromogenic
delay of ET assay (day)	1	1	1
detection limit of ET assay	10 pg/ml	5 pg/ml	10 pg/ml
Characteristics of population			
severe sepsis (%)	100	100	100
septic shock (%)	100	49	51.7
immunodepression (%)	61	49	4.3
GNB infection (%)	Na	49	40.5
Results			
incidence of positive ET (%)	43	47	51.2
mean value of ET (pg/ml)	440 ± 120	60.5 ± 16.5	310 ± 810
Endotoxin correlation			
organ dysfunction	yes	yes	no
lactates level	yes	yes	no
hemodynamic data or shock	yes	yes	no
GNB infection	no	no	no
death	no[a]	no	no

[a] except for the subgroup of patients with positive endotoxemia and bacteremia in which the mortality was 39%, versus 7% in the subgroup with positive bacteremia but without endotoxemia ($p = 0.034$).

resistance and multiple organ failure. One of the major reasons for this difference could be related to the population studied. In fact, even if the size of the population and the incidence of endotoxaemia were similar (Table 3), only patients with septic shock were included in the study of Danner and colleagues [10] and more than 50% of the patients analysed in these two studies presented immunodepression on admission. In our study, no patient had AIDS, chemotherapy or haematological cancer and only five patients (4.3%) could be considered as immunocompromised because of steroid treatment. In this infectious disease setting this immunodepression variability could be of particular importance, account for the discrepancy and hinder the exact comparison of the endotoxin prognostic role [16].

The key to the management of septic patients is to provide them with an adapted early antibiotherapy [17]. In our study, endotoxaemia was associated with GNB infection only in cases of bacteraemic infection irrespective of the site of sepsis. So, the bacteriological interest of endotoxin assay for early antibiotherapy adaptation in a general population admitted to the ICU appears to be limited. This lack of predictive value was also observed in several studies using the same LAL assay [18]. However, according to some previous studies [19, 20], there was a trend for the association between endotoxaemia and GNB bacteraemia and GNB abdominal and urinary sepsis. The small number of patients could account for the non-statistical significance. Because of the small number of patients with meningococcal meningitis ($n = 3$), we could not compare our results with previous studies [8]. In the same way, we could not discuss the interest of endotoxin detection in immunocompromised septic patients.

Limits

To assess the relationship between an endotoxin LAL assay and sepsis precisely, our study had some limits: heterogeneous population including medical and surgical diseases, a majority of pulmonary infection which could explain the absence of association between endotoxaemia and GNB infection, because pulmonary infections are not frequently associated with GNB infection, and more rarely bacteraemic, GNB-related infection in only about 50% of the cases, previous use of antibiotics and the inclusion of non-documented infection. The choice of a single endotoxin assay was also debatable because of the short half-life of endotoxin. It could influence the results, but in our study, like in Guidet's [11] endotoxin clearance appeared to be slow. All these factors could be biases decreasing the accuracy of the endotoxin assay's prognostic value but, on the other hand, our patients were more representative of a ICU general population according to the French multicentric registry [21], and our study provides more precise information about the relationship between endotoxin and outcome in the current clinical management of severe sepsis in ICUs.

Moreover, the only quantitative assay currently available for endotoxin detection is the LAL assay. This indirect method of endotoxin detection is problematic and complex, involving several different enzymes [22, 23]. The unreliability of the LAL assay is probably important in order to explain the lack of predictive value of the endotoxin in human studies [24]. In three studies [24, 25, 26] detection of the endotoxin by a rapid qualitative assay based on visible agglutination of red blood cells with monoclonal antibody on their surface in the presence of the endotoxin [24] or by a chemoluminescence method [25, 26] more reliably predicted hospital outcome in patients admitted to ICUs, in comparison to the LAL method. These results had to be validated in specific septic populations (in these three studies only 18.9 % [24], 32 % [25] and 25 % [26] of the patients had suspected or proven sepsis). So, even if several animal and clinical studies have questioned the significance of a circulating endotoxin in predicting the severity and the lethality of infections and its leading part in the occurrence of septic shock and organ dysfunctions, it would be necessary to re-examine the problem of endotoxin detection by LAL assay and to test other techniques before reconsidering its pathogenesis.

Conclusions

The early presence of endotoxaemia, as detected by an LAL assay in ICU patients with severe sepsis and septic shock, was not associated with mortality and the occurrence of organ dysfunction. Moreover, endotoxaemia detected by a LAL assay had a GNB infection predictive value only in the case of bacteraemia. Because of LAL assay technical problems and/or because of confounding factors in clinical practice, the use of endotoxaemia as a diagnostic or a prognostic marker in daily practice remains difficult and does not seem to be useful for the general management of unselected non-immunocompromised patients with severe sepsis in ICUs.

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