

## Full Text



### Natural Cytokine Antagonists and Endogenous Antiendotoxin Core Antibodies in Sepsis Syndrome

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#### Abstract

**Objective:** To assess the value of measuring circulating concentrations of mediators (endotoxin, tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and interleukin-6 (IL-6)) and their endogenous antagonists (antiendotoxin core antibody (EndoCab), interleukin-1 receptor antagonist (IL-1ra), and soluble TNF receptors (sTNF-R)) in predicting mortality and organ failure in sepsis syndrome.

**Design:** Cohort study with a follow-up period of 30 days.

**Setting:** Intensive therapy units of five tertiary referral centers in Scotland.

**Subjects:** A total of 146 intensive therapy unit patients with sepsis syndrome underwent repeated sampling during a 10-day period following admission to an intensive therapy unit.

**Main Outcome Measures:** Circulating concentrations of mediators and antagonists were compared in survivors and nonsurvivors.

**Results:** Median Acute Physiology and Chronic Health Evaluation II score was 23 (range, 8 to 40). Mortality at 30 days was 49%. On entry to the study, circulating endotoxin was detected in 66% of patients, TNF-alpha in 14%, and IL-1beta in 29%. Levels did not predict mortality or organ failure. Patients with IL-6 concentrations in excess of 3000 pg/mL had an increased mortality rate (64% vs 40%,  $P=.02$ ). The incidence of IgG EndoCab depletion on entry to the study was 26% in nonsurvivors and 10% in survivors ( $P=.02$ ). Initial concentrations of both type I and type II sTNF-R were significantly higher in nonsurvivors ( $P<.01$ ). Initial circulating IL-1ra concentrations were not of value in predicting mortality. Cytokine antagonists were present in concentrations 30- to 100 000-fold greater than their corresponding cytokine.

**Conclusion:** The observed high circulating levels of the cytokine antagonists IL-1ra and sTNF-R and the relatively small proportion of patients developing EndoCab depletion may contribute to the limitations of therapies that aim to augment natural defenses against endotoxin or the proinflammatory cytokines. (JAMA. 1995;274:172-177)

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Severe sepsis is a major cause of death in patients admitted to the intensive therapy unit (ITU) and continues to have a high mortality (25% to 58%) despite appropriate surgery, potent antibiotics, and intensive supportive therapy [1-

4]. It is thought that sepsis syndrome results from activation of the host's defense mechanisms in response to invading microorganisms and their products (eg, endotoxin) [5]. Recent investigations have focused on aspects of the proinflammatory cytokine network, believed to be of central importance in the reaction to bacteria and endotoxin. Tumor necrosis factor-alpha (TNF-alpha) and interleukin-1beta (IL-1beta) are thought to be early mediators of the response to microbiological invasion. In addition to the proinflammatory mediators present in sepsis, natural antagonists have been identified, such as endogenous antiendotoxin core antibody (EndoCAB), interleukin-1 receptor antagonist (IL-1ra), and soluble TNF receptors (sTNF-R). Although evidence of mediator activation is abundant, our understanding of the interrelationships between each mediator and its antagonist in clinical sepsis is poor.

In 1991, Zeigler et al [3] reported a 39% reduction in the mortality of patients with gram-negative bacteremia treated with HA-1A, a human monoclonal antibody against the lipid A moiety of Escherichia coli J5 endotoxin. However, there was no improvement in survival when all patients who were randomized were included in the analysis. The subgroup of patients who appeared to benefit from administration of HA-1A could only be identified retrospectively when blood culture [3] or endotoxin results [6] became available. Despite evidence that circulating levels of mediators predict outcome [7-12], it remains difficult to identify prospectively the patients who might benefit from proinflammatory antagonists [1,13,14]. A major factor that confounds the use of proinflammatory markers to identify those who would benefit from exogenous antagonists is that this approach does not take into account that the endogenous pool may already be replete.

In an attempt to develop a predictive model and evaluate the significance of the proinflammatory and anti-inflammatory events in sepsis syndrome, we have compared the circulating levels of a number of proinflammatory and anti-inflammatory components in survivors and nonsurvivors.

## METHODS

### Patients

A group of 146 ITU patients with sepsis syndrome were studied during a 16-month period in five ITUs in Edinburgh (Western General Hospital and Royal Infirmary) and Glasgow (Western Infirmary, Victoria Infirmary, and Stobhill Hospital), Scotland. Patients were enrolled if there was clinical suspicion of sepsis and they fulfilled the following criteria of sepsis syndrome [15]: (1) hyperthermia (>38 degrees C), hypothermia (<35.5 degrees C), or a proven site of infection; (2) tachycardia (>90 beats per minute in the absence of beta-blockade); (3) tachypnea (>20 breaths per minute) or a requirement for mechanical ventilation; and (4) evidence of dysfunction of one or more end organs, defined as: (a) plasma lactate greater than 1.2 mmol/L, base deficit greater than 5 mmol/L, or systemic vascular resistance less than 800 dyne x s x cm<sup>-5</sup>; (b) PaO<sub>2</sub>/fraction of inspired oxygen less than 30 kPa or PaO<sub>2</sub> less than 9.3 kPa; (c) less than 120-mL urine output during 4 hours; or (d) Glasgow Coma Scale score less than 15 in the absence of a neurological lesion. These criteria represent a slight modification of those defined by Bone and coworkers [2] to allow inclusion of patients with a proven infection who were in a phase between hypothermia and hyperthermia.

Approval for the study was granted by each center's ethical committee and written informed consent obtained from the patient or a relative. Patients were treated by standard methods.

Patients were followed up until they died or for 30 days. Severity of illness was scored on admission to the study using the Acute Physiology and Chronic Health Evaluation (APACHE) II system [16]. Organ failure was assessed daily for 7 days and at day 10 using an organ failure score [17].

### Blood Sampling

Blood sampling was carried out twice in the first 24 hours following admission to the study, daily thereafter for 7 days, and on day 10 by venipuncture or from an indwelling arterial line. Blood (20 mL) was collected for aerobic and anaerobic culture at both time points in the first 24 hours of the study. Blood for endotoxin and EndoCAB measurement was withdrawn into a gel barrier heparinized endotoxin-free tube (Kabi tube (Chromogenix), Quadrantech, Epsom, England), centrifuged at 2000 rpm for 10 minutes, and stored at -20 degrees C until analysis. Blood for cytokine measurement was withdrawn into a glass tube, allowed to clot at room temperature, and centrifuged at 2000 rpm for 10 minutes. The resulting serum was stored at -20 degrees C until analysis.

### Endotoxin and EndoCAB Assays

Plasma endotoxin concentrations were determined using a kinetic limulus amoebocyte lysate assay (Chromogenic Coatest Endotoxin (Chromogenic) kit, Quadratech, Surrey, England) according to the manufacturer's instructions. Plasma samples were assayed at a 1:10 dilution after heat inactivation at 85 degrees C for 15 minutes. The assay was sensitive to levels of endotoxin greater than 0.05 endotoxin units (EU)/mL.

The IgG and IgM EndoCAb were measured with an enzyme-linked immunosorbent assay (ELISA) as previously described [18]. Briefly, an equimolar mixture of four Rc lipopolysaccharides (one each from E coli, Pseudomonas aeruginosa, Salmonella typhimurium, and Klebsiella pneumoniae) was used in the solid phase. A standard curve was set up using a calibrated reference serum with a high-titer of IgG or IgM EndoCAb. Bound IgG or IgM EndoCAb was detected with alkaline phosphatase anti-IgG or anti-IgM (heavy-chain specific). Interassay variability was 6%. Depletion of EndoCAb was defined as a level less than the 10th percentile of a group of healthy adult blood donors (n=1014). The 10th percentile for IgG EndoCAb was 33.7 IgG median units (GMU)/mL and for IgM EndoCAb was 39.7 IgM median units (MMU)/mL, where median units are a percentage of the respective IgG and IgM median levels in healthy adult blood donors. Total IgG and IgM were measured by the standard laboratory method of laser nephelometry [19].

### Cytokine Assays

TNF-alpha was measured with an indirect ELISA using a murine monoclonal antihuman TNF-alpha antibody and peroxidase-conjugated Fab fragments of a murine monoclonal antihuman TNF-alpha antibody, as described by the manufacturers (Boehringer-Mannheim, Lewes, England). A standard curve was constructed using recombinant human TNF-alpha (British Biotechnology, Abingdon, England). The lower limit of detection of the assay was 15 pg/mL. Recovery of recombinant TNF-alpha added to serum from septic patients with low TNF-alpha levels was 68% and compared with the 70% quoted for normal serum in the manufacturer's protocol. Interassay variation was 7%.

IL-1beta and IL-1ra were measured by ELISA (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. The lower limit of detection for IL-1beta was 3.9 pg/mL. Recovery of recombinant IL-1beta from serum was 95%, and interassay variation was 8%. The lower limit of detection for IL-1ra was 48 pg/mL. Recovery of recombinant IL-1ra from serum was 99%, and interassay variation was 7%. IL-1beta and IL-1ra were measured at the first time point only. Data for these cytokines were unavailable in six patients.

ELISA for IL-6 used a murine monoclonal antihuman IL-6 antibody (Boehringer-Mannheim, Lewes, England) as the capture antibody and a goat polyclonal antihuman IL-6 antibody (British Biotechnology, Abingdon, England) followed by a peroxidase-conjugated antigoat IgG (Sigma UK Ltd, Poole, England) to detect bound IL-6. A standard curve was constructed using recombinant human IL-6 (British Biotechnology, Abingdon, England). The lower limit of detection of the assay was 75 pg/mL. Recovery of recombinant IL-6 added to serum from septic patients with elevated IL-6 levels was 80%. Interassay variation was 4%.

Type I and type II sTNF-R were detected by sandwich ELISA, using monoclonal and polyclonal anti-sTNF-R55 and anti-sTNF-R75 antibodies. Purified sTNF-R55 and sTNF-R75 were used to construct standard curves. The lower limit of detection of the assay was 0.39 ng/mL for both receptors. Recovery of sTNF-R55 and sTNF-R75 added to serum from septic patients with elevated receptor levels was 80% and 90%, respectively. Interassay variation was 10% and 3%, respectively.

### Statistics

Results are presented as the median and range. Group frequencies were compared using the chi squared ( $\chi^2$ ) test with continuity correction. Continuous data were compared using the Mann-Whitney U test and Spearman rank correlation analysis. Stepwise logistic regression analysis was used to determine which mediators best predicted mortality. Results were considered to be statistically significant for values of  $P < .05$ .

### RESULTS

In the 146 patients studied, the 30-day mortality rate was 49%. When weightings for diagnosis were included, this result compares with a predicted mortality rate of 50.5%, calculated using the APACHE II model [16]. Patient demographics are shown in Table 1. Survivors were significantly younger than nonsurvivors ( $P < .001$ ) and had a significantly lower median APACHE II score ( $P < .001$ ). Similarly, maximum daily organ failure scores in the 10-day study period were higher in nonsurvivors ( $P < .001$ ). Sepsis from abdominal infection was diagnosed in 64 patients at

laparotomy. Respiratory sepsis, diagnosed by clinical signs, x-ray changes, or positive sputum culture results, was present in 42 patients. Meningococcal sepsis with detectable circulating antigen was diagnosed in five patients. Thirty-four patients had miscellaneous or unidentified sources of sepsis. In only one patient was the urinary tract identified as the primary source of sepsis. Patients were admitted to the study at a median of 1.9 days following the onset of their illness (range, 0.1 to 31.7 days).

Characteristic	Overall	Survivors	Nonsurvivors	P	z or $\chi^2$
No. of patients	146	75	71	...*	...
Median (range) age, y	63 (16-88)	56 (17-84)	68 (16-86)	<.001†	-3.34
Male:female	74:72	22:42	41:30	.13‡	2.2
Median (range) admission APACHE II score§	23 (8-40)	19 (8-39)	24 (8-40)	<.001†	-3.53
Median (range) organ failure score	5 (1-10)	5 (1-9)	7 (1-10)	<.001†	-4.38

\*Ellipses indicate not applicable.  
†Mann-Whitney U test.  
‡ $\chi^2$  test.  
§APACHE indicates Acute Physiology and Chronic Health Evaluation.  
||Organ failure score is the maximum value recorded for each patient during the 10-day study period.

Table 1. Demographic Data

At the time of admission to the study, 53 patients were in shock, as defined by a systolic blood pressure of less than 90 mm Hg or a decrease of greater than 40 mm Hg in a previously hypertensive patient. An additional 36 patients had documented evidence of shock before entry into the study. The presence of shock on or before entry was associated with a significantly higher mortality rate (58% vs 34%, chi squared ( $\chi^2$ ) = 6.9,  $P$  = .008).

A total of 26 patients (18%) had positive blood culture results before or during the first 24 hours of the study. Gram-negative organisms were isolated from 12 patients (8%), gram-positive from 14 (10%), and yeasts from two patients. Coagulase-negative staphylococci were isolated in three patients. One of these patients had a concomitant streptococcal bacteremia and another had bacteroides isolated from his blood. In these two cases coagulase-negative staphylococcus may have been a contaminant. However, in the final patient the underlying problem was bacterial endocarditis for which coagulase-negative staphylococcus is a relevant organism.

### Endotoxin and Antiendotoxin Antibodies

At the time of admission to the study, 96 patients (66%) had detectable plasma endotoxin (median, 1.20 EU/mL; range, 0.05 to 312.0 EU/mL). Levels of endotoxin did not predict mortality Table 2 and showed no correlation with maximum daily organ failure scores calculated during the 10-day study period ( $r$  = 0.03,  $P$  = .4). During the 10-day study period, 125 patients (86%) had detectable plasma endotoxin at one or more time points.

Mediator*	Median (Range)			P†	z or $\chi^2$
	Overall	Survivors	Nonsurvivors		
Endotoxin, EU/mL	0.36 (<0.05-312.0)	0.38 (<0.05-312.0)	0.33 (<0.05-166.0)	.81	-0.24
IgG EndoCAB, GMU/mL	93.1 (8.3-1156.7)	103.3 (9.8-710.8)	78.6 (8.3-1156.7)	.14	-1.46
IgM EndoCAB, MMU/mL	49.9 (<5.2-367.7)	57.4 (<5.2-367.7)	43.6 (<5.2-261.2)	.01	-2.52
TNF- $\alpha$ , pg/mL	<15 (<15-444.5)	<15 (<15-444.5)	<15 (<15-101.1)	.92	-0.11
Interleukin-1 $\beta$ , pg/mL	<3.9 (<3.9-355.3)	<3.9 (<3.9-355.3)	<3.9 (<3.9-154.0)	.53	-0.62
Interleukin-6, pg/mL	1185.6 (<75-453 125)	1103.6 (<75-62 570)	1491.5 (<75-453 125)	.09	-1.68
sTNF-R55, ng/mL	12.3 (3.1-68.6)	10.8 (3.1-36.6)	13.8 (4.5-68.6)	.001	-3.27
sTNF-R75, ng/mL	12.8 (1.9-83.3)	10.7 (1.9-47.2)	13.9 (4.0-83.3)	.01	-2.58
Interleukin-1 receptor antagonist, ng/mL	17.0 (0.2-1115.1)	15.9 (0.3-500.4)	19.4 (0.2-1115.1)	.95	-0.06

\*EU/mL indicates endotoxin units per milliliter; GMU/mL, IgG median units per milliliter; MMU/mL, IgM median units per milliliter; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; and sTNF-R, soluble tumor necrosis factor receptor.  
†Mann-Whitney U test.

Table 2. Plasma Endotoxin and Antiendotoxin Core Antibody (EndoCAB) and Serum Cytokine and Antagonist Levels on Entry to the Study

Initial plasma IgG EndoCAB and IgM EndoCAB concentrations are shown in Table 2. The median concentration of IgM EndoCAB in nonsurvivors was significantly lower than in survivors ( $z=-2.52$ ,  $P=.01$ ), but the distribution of levels within the two groups showed considerable overlap. Initial plasma IgG EndoCAB concentrations were not significantly different in survivors and nonsurvivors Table 2. EndoCAB concentration showed no correlation with organ failure scores (IgG:  $r=0.03$ ,  $P=.4$ ; IgM:  $r=-0.12$ ,  $P=.08$ ). Depletion of IgG EndoCAB (defined as a level below the 10th centile of a normal population) occurred in 26 patients, 18 of whom died (chi squared ( $\chi^2$ ) =4.4,  $P=.02$ ), resulting in a positive predictive value of 69%. Patients with IgG EndoCAB depletion on entry to the study had significantly higher levels of endotoxin (median, 2.3 EU/mL; range, 0 to 166.0 EU/mL) than those in whom IgG EndoCAB was not depleted (median, 0.27 EU/mL; range, 0 to 312.0 EU/mL;  $z=-3.1$ ,  $P=.002$ ). IgM EndoCAB depletion occurred in 55 patients (38%), 32 (58%) of whom died, but did not predict mortality (chi squared ( $\chi^2$ ) =2.6,  $P=.1$ ). During the 10-day sampling period, IgG EndoCAB depletion occurred in 38 patients (26%) at one or more time points, and IgM EndoCAB depletion occurred in 121 patients (83%).

Patients with IgM EndoCAB depletion on entry to the study were significantly older (median age, 69 years; range, 16 to 83 years) than those who did not have IgM EndoCAB depletion (median age, 62 years; range, 16 to 86 years;  $z=-2.8$ ,  $P=.005$ ). There were significant correlations between total immunoglobulin and EndoCAB concentrations (IgG:  $r=0.5$ ,  $P<.001$ ; IgM:  $r=0.65$ ,  $P<.001$ ). However, total immunoglobulin levels did not predict mortality (IgG:  $z=-0.51$ ,  $P=.6$ ; IgM:  $z=-1.25$ ,  $P=.2$ ).

### Cytokines and Endogenous Antagonists

Serum cytokine levels on entry to the study are shown in Table 2. TNF-alpha was detected in only 21 patients (median, 34.6 pg/mL; range, 16.7 to 444.5 pg/mL), was of no value in predicting mortality, and did not correlate with organ failure scores ( $r=0.07$ ,  $P=.2$ ). However, TNF-alpha was detected in 25% of patients who were in shock at the time of sampling, compared with 9% of those who were not in shock (chi squared ( $\chi^2$ ) =5.7,  $P=.02$ ). Patients with detectable TNF-alpha were admitted to the study a median of 1.2 days (range, 0.1 to 8.1 days) after the onset of their illness. Those in whom TNF-alpha was undetectable were admitted significantly later, at a median of 2.1 days (range, 0.1 to 31.7 days;  $z=-2.2$ ,  $P=.03$ ). An additional 15 patients had detectable TNF-alpha at other time points in the study.

Type I and type II sTNF-R were detected in all serum samples taken during the first 24 hours of the study. There was a positive correlation between concentrations of sTNF-R55 and sTNF-R75 ( $r=0.81$ ,  $P<.001$ ). Nonsurvivors had significantly higher circulating concentrations of both soluble receptors on entry to the study Table 2, as did patients with shock on entry to the study Table 3. Levels of sTNF-R were found to correlate positively with plasma creatinine (sTNF-R55:  $r=0.62$ ,  $P<.001$ ; sTNF-R75:  $r=0.63$ ,  $P<.001$ ). Detection of TNF-alpha in the initial sample was associated with significantly higher circulating concentrations of sTNF-R75. Those with detectable TNF-alpha had a median sTNF-R75 concentration of 18.1 ng/mL (range, 3.3 to 83.3 ng/mL), while those in whom TNF-alpha was not detectable had a

median concentration of 11.9 ng/mL (range, 1.9 to 43.2 ng/mL;  $z=-2.5$ ,  $P=.01$ ). The ratio of TNF-alpha to sTNF-R was not of value in predicting mortality ( $z=-0.63$ ,  $P=.52$ ). sTNF-R was present in concentrations approximately 300-fold greater (range, 30 to 1300) than TNF-alpha.

Mediator*	Median (Range)		P†	z or $\chi^2$
	Shock on Entry	Not Shocked		
Endotoxin, EU/mL	0.53 (<0.05-312.0)	0.27 (<0.05-166.0)	.27	-1.18
IgG EndoCAb, GMU/mL	116.9 (12.3-1156.7)	89.3 (8.3-950.6)	.25	-1.16
IgM EndoCAb, MMU/mL	49.7 (5.2-367.7)	50.0 (5.2-299.8)	.66	-0.44
TNF- $\alpha$ , pg/mL	<15 (<15-444.5)	<15 (<15-111.2)	.007	-2.69
Interleukin-1 $\beta$ , pg/mL	<3.8 (<3.9-355.3)	<3.9 (<3.9-90.9)	.004	-2.85
Interleukin-6, pg/mL	2641.8 (<75-453 125)	1103.6 (<75-88 017)	.003	-2.94
sTNF-R55, ng/mL	14.0 (4.1-68.6)	11.0 (3.1-36.6)	.001	-4.09
sTNF-R75, ng/mL	16.8 (4.5-83.3)	9.9 (1.9-24.8)	<.001	-3.31
Interleukin-1 receptor antagonist, ng/mL	27.8 (0.6-1115.1)	13.9 (0.2-349.7)	.004	-2.93

\*EU/mL indicates endotoxin units per milliliter; GMU/mL, IgG median units per milliliter; MMU/mL, IgM median units per milliliter; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; and sTNF-R, soluble tumor necrosis factor receptor.  
†Mann-Whitney U test.

Table 3. Plasma Endotoxin and Antiendotoxin Core Antibody (EndoCAb) and Serum Cytokine and Antagonist Levels on Entry to the Study in Shock

IL-1beta levels on entry to the study are shown in Table 3. IL-1beta was detected in 41 of 140 patients (median, 9.0 pg/mL; range, 4.0 to 355.3 pg/mL), but was not of predictive value for mortality and did not correlate with organ failure scores ( $r=0.14$ ,  $P=.06$ ). IL-1beta was detected in 44% of patients in shock at the time of sampling, compared with 21% who were not in shock (chi squared ( $\chi^2$ ) = 7.1,  $P=.008$ ). Patients with detectable IL-1beta were admitted earlier (median, 1.4 days; range, 0.1 to 28.5 days) in the course of their illness compared with those who did not have detectable IL-1beta (median, 2.4 days; range, 0.1 to 31.7 days;  $z=-2.8$ ,  $P=.006$ ).

Serum IL-1ra was detected in all patients on entry (median, 17.0 ng/mL; range, 0.2 to 1115.1 ng/mL), but concentrations were not of predictive value with respect to mortality Table 2. As with sTNF-R, patients in shock at the time of sampling had significantly higher serum concentrations of IL-1ra Table 3. There was also a small but significant correlation between IL-1ra and creatinine concentrations ( $r=0.2$ ,  $P=.015$ ). Patients with detectable IL-1beta had significantly higher serum concentrations of IL-1ra (median, 71.8 ng/mL; range, 2.5 to 1115.1 ng/mL), compared with those in whom IL-1beta was undetected (median, 12.7 ng/mL; range, 0.2 to 249.7;  $z=-5.5$ ,  $P<.001$ ). In addition, there was a positive correlation between levels of IL-1beta and IL-1ra ( $r=0.44$ ,  $P=.001$ ), but the ratio of IL-1beta to IL-1ra was not of value in predicting mortality ( $z=-0.37$ ,  $P=.7$ ). IL-1ra was present in concentrations greatly in excess of IL-1beta (median ratio, 3700; range, 50 to 106 500).

Serum IL-6 was detected in concentrations up to 0.5 micrograms/mL. Initial levels of IL-6 were not of value in predicting mortality Table 2, but did have a small correlation with organ failure scores ( $r=0.27$ ,  $P=.001$ ). Although individual levels were not of predictive value, the 44 patients with initial IL-6 concentrations of greater than 3000 pg/mL had a significantly higher mortality rate than the 102 patients with concentrations of less than 3000 pg/mL (64% vs 42%, chi squared ( $\chi^2$ ) = 4.8,  $P=.03$ ). Patients in shock on entry to the study had higher circulating IL-6 concentrations than those who were not in shock Table 3. Detection of IL-1beta was associated with significantly higher circulating concentrations of IL-6 on entry to the study (median, 6276.8 pg/mL; range, 128.9 to 453 125.0 pg/mL vs median, 749.0 pg/mL; range, <75 to 58 062.5 pg/mL;  $z=-6.6$ ,  $P<.001$ ). The majority of patients displayed a similar pattern of circulating IL-6 during the study, with the highest values occurring in the first 24 to 48 hours of the study and levels declining thereafter. This pattern was observed in both survivors and nonsurvivors. However, the rate of decline varied depending on outcome, with evidence of a sustained mediator response in nonsurvivors. On day 7 of the study, 17 of 23 eventual nonsurvivors had detectable IL-6, compared with 17 of 41 survivors (chi squared ( $\chi^2$ ) = 4.6,  $P=.03$ ), and on day 10, 13 of 15 nonsurvivors had detectable IL-6, compared with only nine of 41 survivors (chi squared

( $\chi^2$ ) = 16.7,  $P < .001$ ).

A stepwise logistic regression analysis was performed to determine which of the mediator variables in Table 2 were predictive of mortality. Only sTNF-R55 was found to significantly predict mortality ( $P = .006$ ). No other variable was of sufficient predictive value to gain entry into the regression model.

## COMMENT

Given the size and multicenter nature of the present study, it is likely that the population examined is representative of patients admitted with sepsis syndrome to ITUs in the United Kingdom. The number of patients with urinary tract sepsis (less than 1%) is much lower than in recent US series (11% to 14%) [1-3]. While the urinary tract was the most commonly identified source of bacteremia in a United Kingdom hospital population [4], these patients had a relatively low mortality rate (9.8%), and many would not have been considered for admission to ITUs. The paucity of patients with urinary tract sepsis may explain the low incidence of positive blood culture results observed in the present study. Such heterogeneity in patient populations may contribute to the different mortality rates and therapeutic effects observed when novel agents are used to treat sepsis.

The criteria that we used to define sepsis syndrome have been used on a number of occasions to identify ITU patients for study. In such patients, circulating endotoxin and cytokine levels have been observed to be predictive of severity of illness and mortality [20,21]. In the present study, plasma endotoxin was detected in 66% of patients on entry and in 86% during the study, although circulating levels did not predict mortality. There are several possible explanations for this finding.

First, systemic endotoxemia is believed to be intermittent, and its true extent may not be reflected by once- or twice-daily sampling. The half-life of circulating endotoxin in experimental models of sepsis is short [22], suggesting that an ethically unacceptable frequency of blood sampling would be required to detect all episodes of endotoxemia. Second, antibiotic administration increases the release of endotoxin from bacteria [23], and the majority of our patients received antibiotics before entering the study. The limulus amebocyte lysate assay involves heat inactivation, which may denature proteins bound to endotoxin. The results obtained, therefore, may not give a true reflection of endotoxin's bioactivity. Furthermore, it has recently become apparent that recognition of endotoxin by the host involves several different receptor mechanisms [24], not all of which result in a biological response. Variations in the relative availability of these receptors may alter the response to endotoxin. Augmentation of the response to endotoxin by lipopolysaccharide-binding protein, an acute phase reactant, could be one such source of variation. Together, these factors limit the predictive value of endotoxin measurement.

Endogenous antiendotoxin antibody levels may reflect exposure to endotoxin, with a reduction in antibody titer reflecting consumption in the short term and a rise in titer reflecting synthesis of new antibody by an activated immune system. Attention has recently focused on the use of such antibodies against the core region of endotoxin in the treatment of a variety of gram-negative infections. In support of this strategy, Pollack et al [25] have found increased levels of circulating antibodies to *E. coli* J5 in survivors of *P. aeruginosa* septicemia. Moreover, reduced serum levels of IgG antibodies to the endotoxin core of *Salmonella* minnesota R595 have been associated with an increased mortality in patients with sepsis [20,26]. In the present study, circulating antibodies were detected by binding to lipopolysaccharides from four genera of bacteria. These measurements should provide a more representative index of endotoxin exposure from a variety of sources. EndoCAB depletion (ie, a level less than the 10th percentile) was associated with significantly higher endotoxin levels and those patients with IgG EndoCAB depletion on admission to the study had a significantly higher mortality. IgG EndoCAB depletion, therefore, allowed identification of a high-risk group of patients with sepsis syndrome. However, these individuals represented only 26% of nonsurvivors, and therefore depletion of this endogenous EndoCAB pool cannot be the dominant factor determining outcome.

Circulating cytokine concentrations, as a representation of the host response in sepsis syndrome, have been reported to be of predictive value. Higher TNF- $\alpha$  levels have been detected in nonsurvivors of septic shock [8,27] and meningococcal sepsis [11]. In our heterogeneous group of patients, TNF- $\alpha$  was detected in only 14% of patients on entry to the study and was not found to be of value in predicting mortality. The wide variation in detection rates of TNF- $\alpha$  may be explained by the variety of assay methods used and by the timing of sampling. Some assays may detect bound TNF- $\alpha$ , which is not thought to be biologically active. TNF- $\alpha$  is thought to appear early in the

course of sepsis, and indeed in the present study, patients with detectable TNF-alpha on entry to the study were admitted significantly earlier in the course of their illness. Detection of TNF-alpha was also associated with the presence of shock at the time of sampling, suggesting that circulating TNF-alpha may be present in more severely ill patients. This observation could represent an overflow of the cytokine from sites of inflammation, and it may be that tissue levels would provide more information regarding the severity of sepsis. This is not an area that can be studied with ease in the clinical situation.

Soluble receptors have been identified for a number of cytokines, including TNF-alpha. They represent shed portions of cell-surface receptors, and two such soluble receptors exist for TNF-alpha (sTNF-R55 and sTNF-R75). Endotoxin and other stimuli cause down-regulation of surface TNF receptors [28], and administration of endotoxin to humans results in release of sTNF-R into the circulation [29]. The protective effect of exogenous sTNF-R in lethal endotoxemia in mice has been demonstrated [30]. Down-regulation of surface receptors, reducing cell responsiveness, may be accompanied by shedding of soluble receptors, which could act as a buffer for free TNF-alpha in the circulation. Increased levels of sTNF-R in sepsis have recently been reported [31,32]. In the present study, higher circulating concentrations of both sTNF-R55 and sTNF-R75 were found in nonsurvivors. The correlation between concentrations of creatinine and sTNF-R suggests that excretion of sTNF-R is dependent on renal function. This dependence may have contributed to the higher serum levels observed in nonsurvivors. Soluble TNF-R was found in concentrations 30- to 1000-fold higher than those of TNF-alpha, concentrations at least as high as those seen in experimental sepsis [29]. Further studies are required to determine the significance of these shed receptors as a defense mechanism in human sepsis.

Circulating IL-1beta has been detected in varying amounts depending on the assay method used. In the present study, IL-1beta was detected in 27% but had no predictive value with respect to mortality. As with TNF-alpha, IL-1beta was detected more frequently in patients with shock. In addition, patients with detectable IL-1beta were admitted to the study earlier in the course of their illness, reflecting its role as an early mediator in major sepsis.

IL-1ra, the naturally occurring antagonist of IL-1, is currently under investigation as an adjunctive therapy in the management of sepsis [13]. In experimental endotoxemia, circulating IL-1ra is detected in 100-fold greater concentrations than IL-1beta [33]. IL-1 receptor occupancy of approximately 5% can induce a biological response, suggesting that the concentrations of IL-1ra that occur in sepsis could have a modulating effect. In the present study, IL-1ra concentrations measured on entry to the study were not of value in predicting mortality. Patients with detectable IL-1beta had significantly higher concentrations of IL-1ra, but ratios of IL-1beta to IL-1ra were not of value in predicting mortality. The median concentrations of IL-1ra were 3700 times greater than those of IL-1beta. At such levels, it is not known whether administration of exogenous IL-1ra would have any additional value in modulating the proinflammatory response. This finding is supported by the results of recent clinical studies of IL-1ra administration, as reported in the press.

Circulating IL-6 has also been reported to be of predictive value in sepsis, with values between greater than 1000 pg/mL and greater than 7000 pg/mL being associated with increased mortality [34,35]. In the present study, 3000 pg/mL was selected as a representative cutoff, and initial concentrations of IL-6 greater than this value were associated with a higher mortality. IL-6 concentrations were higher in patients who had undergone a period of shock. Higher IL-6 concentrations were also found in patients with detectable IL-1beta on entry to the study. IL-6 was more frequently detected in eventual nonsurvivors on days 7 and 10, supporting the hypothesis of a sustained mediator response [36]. However, the number of nonsurvivors sampled at these time points was small, and therefore the importance of this finding is uncertain. Currently, we have not measured soluble IL-6 receptors. The IL-6 signal is mediated via a specific cell-surface receptor and the signal-transducing molecule, gp130. The soluble IL-6 receptor can also mediate the IL-6 signal through gp130 [37] and therefore cannot be regarded as a cytokine antagonist.

Logistic regression analysis of the concentrations of circulating mediators and their antagonists selected only sTNF-R55 as being significantly predictive of mortality. IgM EndoCAB and sTNF-R75, which were of borderline significance in Mann-Whitney U testing, were not significantly associated with mortality in the multivariate analysis.

The patient group studied herein is broadly similar to those of a number of clinical trials of immunotherapies. Although, in the group as a whole, there were statistically significant differences in the mediator concentrations of



survivors and nonsurvivors, the degree of overlap meant that these were not of value in predicting mortality in individual patients. The balance of proinflammatory and anti-inflammatory cytokines was similar in survivors and nonsurvivors. Depletion of the endogenous IgG antiendotoxin antibody pool (as measured by EndoCAB) was predictive of a high mortality, albeit the majority (74%) of patients had normal circulating levels of these antibodies. These findings cast doubt on the therapeutic strategies currently being pursued in patients with severe sepsis, which aim to augment such natural defenses against endotoxin and the proinflammatory cytokines. Moreover, the best timing of such intervention is not clear. Much further research will be required before the true significance of proinflammatory and anti-inflammatory events in human sepsis is understood and can be harnessed for therapeutic benefit Table 4.

System	Component	SI Reference Interval*	SI Unit	Conversion Factor (Divide by)	Traditional Reference Interval*	Traditional Unit
Plasma	Lactate (as lactic acid)	0.5-2.0	mmol/L	0.1110	5-20	mg/dL

\*These reference values are not intended to be definitive since each laboratory determines its own values. They are provided for illustration only.

Table 4. Conversions From Systeme International (SI) Units to Traditional Units (Modified From The SI Manual in Health Care)

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