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Diagnostic and Prognostic Significance of Plasma Endotoxin Determination in Febrile Patients with Haematological Malignancies

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We evaluated the clinical utility of a new endotoxin-specific chromogenic limulus test in febrile patients with haematological malignancies. The specificity is assured by the removal of factor G, which is sensitive to (1→3)-β-D-glucan, from horseshoe crab amoebocyte lysate. The sensitivity and specificity of the test to systemic gram-negative bacterial infections were 69.7 and 96.3%, respectively. Meanwhile, gram-negative bacteria grew in only 39.7% of endotoxaemic samples. Thus, it seems appropriate to consider gram-negative bacteraemia and endotoxaemia as different entities. Endotoxaemia was significantly associated with septic shock and infectious death, especially in patients with neutropenia. The new test, the results of which are available within 3 h, should help physicians to recognise this ominous sign early and to initiate a prompt countermeasure to endotoxaemia.

Key words: endotoxin, endotoxaemia, haematological malignancy, chromogenic limulus test, (→3)-β-D-glucan, febrile

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INTRODUCTION

ONLY ABOUT one third of pyrexial episodes are usually documented microbiologically in patients with haematological malignancies [1, 2]. Since infection with gram-negative bacteria (GNB) is still one of the major causes of the fever, a method for detecting endotoxin, a common cell wall constituent of GNB, would contribute to its successful treatment. Limulus amoebocyte lysate tests have been used for this purpose with controversial results [3–6]. One reason for this is the lack of specificity due to the use of whole amoebocyte lysate that naturally contains, in addition to endotoxin-sensitive factor C, factor G which is sensitive to (1→3)-β-D-glucan, a major cell wall constituent of fungi [7]. Recently, we succeeded in eliminating factor G activity in the lysate, and developed a new test that is specific to endotoxin (Endospecy, Seikagaku Corporation, Tokyo, Japan) [8]. In this study, we evaluated clinical utility of the test in managing febrile patients with haematological malignancies.

PATIENTS AND METHODS

From 1985 to 1988, blood culture and endotoxin determination were performed on 147 febrile (>38°C) episodes in 103 adult patients with haematological malignancies. The episodes were classified into seven categories according to our modified EORTC classification [2]. Blood was cultured more than once and plasma endotoxin level was determined within 1 h of onset of fever. The recurrence of fever despite continued use of initially effective antibiotics was judged as a different infectious episode. A total of 454 blood samples were cultured (3.1 per episode) and 393 plasma specimens were subjected to Endospecy (2.7 per episode) for endotoxin assay. In addition, 102 infection-free samples were collected to serve as negative controls; 54 before the patients became febrile and 48 after the patients became afebrile.

The following possible risk factors for septic shock and/or

Table 1. Classification of infection and endotoxin determination

Infection	Number of endotoxaemia*/ total (%)	Peak endotoxin level (pg/ml) median (range)
Gram-negative bacteraemia	21/30 (70.0)	13.3 (0–402.6)
Unimicrobial	15/24† (62.5)	8.8 (0–102.9)
Multimicrobial	6/6‡ (100)	154.7 (18.0–402.6)
Gram-positive bacteraemia	1§/11 (9.1)	1.7 (0.3–33.0)
Fungaemia	1¶/5 (20.0)	2.5 (1.2–21.3)
Microbiologically documented infection		
Gram-negative infection	2/3** (66.7)	10.3 (2.8–44.7)
Other infection	3/7†† (42.9)	2.5 (0–21.7)
Clinically documented infection	20/46‡‡ (43.5)	2.7 (0–158.7)
Possible infection	10/36 (27.8)	1.6 (0–85.3)
Doubtful infection	0/9 (0)	1.1 (0–2.7)
Total	58/147 (39.5)	

*The concentration of plasma endotoxin ≥ 3 pg/ml. †*Pseudomonas aeruginosa* (6), *Enterobacter cloacae* (6), *Klebsiella pneumoniae* (3), *Achromobacter xylosoxidans* (2), *Serratia marcescens* (2), *Acinetobacter calcoaceticus* (2), and others (3). ‡Four with *Candida* species and two with gram-positive bacteria. §The patient had severe hepatic failure. ¶The patient had pneumonia and enterocolitis without microbiological proof. **Two pneumonia and one urinary tract infection. ††Fungal pneumonia (5), fungal enterocolitis (1) and pulmonary tuberculosis (1). ‡‡Pneumonia (29), oropharingitis (5), periodontitis (3) and others (9).

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infectious death were recorded: age, sex, underlying disease, disease state, antecedent therapy with antineoplastic agents, antibiotics or corticosteroids, neutropenia (neutrophils < 500/μl), infectious focus, gram-negative bacteraemia, endotoxaemia and septic shock [9]. Prolonged neutropenia that continued for more than 7 days [1] was also evaluated when patients with neutropenia were analysed separately. Standard bacteriological techniques were used; the isolation of gram-positive bacteria belonging to a normal dermal flora in only one culture was judged as contamination. The plasma endotoxin level was deemed normal when it was less than 3 pg/ml according to our previous study with 50 healthy adults [8]; the coefficient of variability of the test was less than 4%. Episodes were considered positive with Endospecy when one or more consecutive samples were abnormal. Data were analysed by Fisher's exact probability test, a forward stepwise logistic regression analysis, and the Wilcoxon–Mann–Whitney test.

RESULTS

Documentation of infections and endotoxin determinations (*Table* 1)

Thirty episodes were associated with gram-negative bacteraemia, 21 of which (70%) were endotoxaemic. The difference in the postive rate of endotoxaemia between uni- and multimicrobial gram-negative bacteraemia was non-significant, but the peak endotoxin levels were significantly higher in the latter than in the former ($P = 0.002$). Of the 11 gram-positive bacteraemias, Endospecy was positive in 1 patient with severe hepatic failure; of the five fungaemias, it was positive in 1 patient with pneumonia and enterocolitis. Of the 10 microbiologically documented infections, two gram-negative pneumonias, two fungal pneumonias and one fungal enterocolitis were positive with the test. The positive rates in the clinically documented and the possible infections were 43.5% (20/46) and 27.8% (10/36), respectively; severe hepatic failure was not seen in any of them. The infection-free samples were all negative. Meanwhile, GNB grew in only 39.7% (23/58) of endotoxaemic samples.

Table 2. Risk factors in relation to septic shock and death

Risk factors	Number of septic shock/total(%)		*P* value*	Number of infectious death/total(%)		*P* value*
Total	16/147	(10.9)		34/136	(25.0)	
Age (years)						
≥60	4/37	(10.8)	NS	13/33	(39.4)	0.038§
<60	12/110	(10.9)		21/103	(20.4)	
Sex						
Male	7/79	(8.9)	NS	14/74	(18.9)	NS
Female	9/68	(13.2)		20/62	(32.3)	
Underlying disease						
Acute leukaemias†	10/90	(11.1)	NS	18/84	(21.4)	NS
Others	6/57	(10.5)		16/52	(30.8)	
Disease states						
Complete remission	5/26	(19.2)	NS	6/26	(23.1)	NS
No remission	11/121	(9.1)		28/110	(25.5)	
Antineoplastic chemotherapy						
Yes	14/119	(11.8)	NS	27/110	(24.5)	NS
No	2/28	(7.1)		7/26	(26.9)	
Antecedent antibiotics						
Yes	9/66	(13.6)	NS	19/60	(31.7)	NS
No	7/81	(8.6)		15/76	(19.7)	
Antecedent corticosteroids						
Yes	10/89	(11.2)	NS	21/83	(25.3)	NS
No	6/58	(10.3)		13/53	(24.5)	
Neutropenia						
Yes	15/101	(14.9)	0.022	26/96	(27.1)	NS
No	1/46	(2.2)		8/40	(20.0)	
Infectious focus						
Yes	8/81	(9.9)	NS	25/75	(33.3)	0.017§
No	8/66	(12.1)		9/61	(14.8)	
Gram-negative bacteraemia‡						
Yes	9/30	(30.0)	0.021§	15/30	(50.0)	0.0007
No	7/117	(6.0)		19/106	(17.9)	
Endotoxaemia						
Yes	12/58	(20.7)	0.003	25/56	(44.6)	0.00002§
No	4/89	(4.5)		9/80	(11.3)	
Septic shock						
Yes				13/16	(81.3)	0.007§
No				21/120	(17.5)	

*Fisher's exact probability test was used to compare differences in proportions. †Blast crises of chronic myelogenous leukaemia were included. ‡Multi-microbial bacteraemias were included. §Also significant by multivariate analysis. NS, non-significant.

The sensitivity of the test was 69.7% (23/33) based on the 30 gram-negative bacteraemias and the three microbiologically documented gram-negative infections. The specificity was 96.3% (129/134) based on the 23 microbiologically documented non-gram-negative infections, nine doubtful infections and 102 infection-free patients as negative controls.

Analysis of risk factors of septic shock and infectious death

The percentages of septic shock and infectious death and the results of univariate analysis of risk factors that might lead to either of the conditions are summarised in Table 2. 11 patients were excluded from the analysis of mortality risks because they died of diseases other than infection (6 of massive haemorrhage and 5 of relentless underlying diseases). In multivariate analysis, the significant risk factor for septic shock was gram-negative bacteraemia ($P = 0.015$), and those for high mortality were advanced age ($P = 0.035$), presence of infectious focus ($P = 0.018$), endotoxaemia ($P = 0.006$) and septic shock ($P = 0.0004$).

In the univariate analysis of the 101 neutropenic episodes, gram-negative bacteraemia and endotoxaemia were significantly associated with septic shock [8/24 (33.3%) versus 7/77 (9.1%), $P = 0.019$ and 12/47 (25.5%) versus 3/54 (5.6%), $P = 0.006$, respectively]; and factors associated with high mortality were prolonged neutropenia [19/48 (39.6%) versus 7/48 (14.6%), $P = 0.011$], gram-negative bacteraemia [13/24 (54.2%) versus 13/72 (18.1%), $P = 0.001$], endotoxaemia [21/47 (44.7%) versus 5/49 (10.2%), $P = 0.0002$], and septic shock [12/15 (80.0%) versus 14/81 (17.3%), $P = 0.006$]. Advanced age showed a borderline significance [8/17 (47.1%) versus 18/79 (22.8%), $P = 0.068$]. In the multivariate analysis, the significant risk factor for septic shock was endotoxaemia ($P = 0.047$) and those for high mortality were advanced age ($P = 0.027$), endotoxaemia ($P = 0.019$) and septic shock ($P = 0.002$). In 50 episodes with prolonged neutropenia, endotoxaemia was even more significantly associated with septic shock and high mortality [11/27 (40.7%) versus 0/23 (0%), $P = 0.0004$ and 16/27 (59.3%) versus 3/21 (14.3%), $P = 0.003$, respectively; the sum of the denominators of the latter is 48, because 2 patients died of non-infectious causes]. Endotoxaemia was also significantly associated with high mortality in the patients with gram-negative bacteraemia [14/21 (66.7%) versus 1/9 (11.1%), $P = 0.014$].

The peak endotoxin levels were significantly higher in episodes with septic shock (median 24.8 pg/ml, range 1.7–402.6) than in those without shock (median 2.2 pg/ml, range 0–184.5, $P = 0.0006$), and also in patients that died (median 19.9 pg/ml, range 0–402.6), compared with those that survived (median 2.0 pg/ml, range 0–158.7, $P = 0.00003$).

DISCUSSION

The specificity of the new endotoxin-specific test, Endospecy, was complete with the samples drawn from the patients when they were afebrile. A few cases of endotoxaemia in non-gram-negative bacterial infections indicate such possibilities as concurrent occult infection with GNB, inadequate handling of endogenous endotoxin by the compromised reticuloendothelial system, and excessive spillage into the systemic circulation of endogenous endotoxin through damaged intestinal wall [10]. The sensitivity to gram-negative bacteraemia was 70%, indicating that not every gram-negative bacteraemia was endotoxaemic. This might have been caused by inactivation of endotoxin by several host defense mechanisms [11–15]. Meanwhile, GNB grew in only 23 out of 58 endotoxaemic samples. Therefore, it seems appropriate to consider gram-negative bacteraemia and endotoxaemia as different entities.

Multivariate analysis revealed that endotoxaemia is significantly associated with death in the whole patient group as well as in the neutropenics. Patients with gram-negative bacteraemia showed a significantly higher death rate when complicated with endotoxaemia. The peak levels of plasma endotoxin were significantly higher in the groups with septic shock or infectious death than those without either condition. Thus, endotoxaemia is definitely a poor prognostic factor in febrile patients with haematological malignancies. Endospecy, with the results being available within 3 h, should help physicians to recognise this ominous sign early and to intitiate a prompt countermeasure such as the use of anti-endotoxin antibodies.

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