Prognostic Value of Endotoxemia in Patients with Gram-Negative Bacteremia Is Bacterial Species Dependent

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Key Words

Endotoxemia · Gram-negative bacteremia · *Escherichia coli* · Sepsis · Septic shock · Limulus assay

Abstract

The prognostic impact of endotoxemia detection in sepsis is unclear. Endotoxemia is detectable in <70% of patients with Gram-negative (GN) bacteremias. Mortality proportion data were available from 27 published studies of patients with GN bacteremia in various settings. Among ten studies restricted to specific types of GN bacteremia, endotoxemia was associated with significantly increased mortality risk for *Neisseria meningitidis* (4 studies; 138 bacteremias; OR 26.0; 95% CI, 1.6–321) but not for *Salmonella enterica* serovar Typhi (3 studies; 36 bacteremias; OR 0.89; 95% CI, 0.01–74.1). For 17 unrestricted studies (319 GN bacteremic patients), endotoxemia was associated with an increased mortality risk with non-*Escherichia coli* Enterobacteriaceae such as *Klebsiella* and *Enterobacter* species (97 bacteremias; OR 3.7; 95% CI, 1.3–10.3). By contrast, *E. coli* (144 bacteremias; OR 0.78; 95% CI, 0.36–1.7), and non-Enterobacteriaceae species such as *Pseudomonas* species (78 bacteremias; OR 1.7; 95% CI, 0.7–4.6) had no increased mortality risk. That endotoxemia de-

tection is predictive of mortality among patients bacteremic with non-*E. coli* Enterobacteriaceae but not *E. coli* is surprising given the presumed commonality of the hexa-acyl lipid A structure among Enterobacteriaceae species.

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Introduction

There is extensive evidence implicating endotoxin in the pathogenesis of Gram-negative (GN) sepsis [1, 2]. Hence, an assay to detect endotoxemia would appear to be a logical adjunct toward targeting novel therapies for sepsis including those specifically directed against endotoxin [3].

However, among large clinical studies, the predictive value of endotoxemia detection remains unclear [4] and a disconnect with animal models exists [5, 6]. On the one hand, studies of patients with plague [7, 8], or meningococemia [9], have found a quantitative correlation between the level of endotoxemia and the degree of organ dysfunction or mortality. On the other hand, larger studies of sepsis in which the type of GN bacteremias were not restricted to any specific GN species type [10–20], have

generated findings that were contradictory. In aggregate, the results from these studies indicate that endotoxemia detection is a borderline predictor of mortality risk (odds ratio <2) [4, 21].

These and some experimental observations [22, 23] raise doubt as to whether the relationship between endotoxemia and outcome is equal for GN bacteremias of different species type. Clarification might also help to reconcile conflicting observations in the clinical literature especially given the variable prevalence of different GN bacteremia species types in different studies [4].

The objective here is to re-examine the prognostic value of endotoxemia in association with bacteremias with *Escherichia coli* versus other species of GN bacteria in the light of four observations. Firstly, mortality occurs less frequently for *E. coli* bacteremias versus other types of GN bacteremias [24–26]. Second, there are specific structural differences in lipid A, the biologically active component of endotoxin (lipopolysaccharide, LPS) for Enterobacteriaceae including *E. coli* versus other GN bacteria such as non-Enterobacteriaceae [1]. There are important discrepancies between the in vivo biological activities of the endotoxins of different types of GN bacteria versus their activities in the limulus assay, which is the most commonly used assay for endotoxemia detection [22, 27, 28]. Finally, endotoxemia is undetectable for >20% of GN bacteremias overall and >40% of *E. coli* [29] bacteremias.

Methods

Data Sources

A computerized search of PubMed (including Medline) was undertaken using the key words ‘endotoxemia’ and ‘limulus’ in the title or abstract and was restricted to studies in humans. This search was supplemented by a hand search for studies reporting mortality outcome data in relation to endotoxemia detection and detection of GN bacteremia with blood culture for patient groups at risk of GN bacteremia. This search has been performed and repeatedly updated [21, 29] between 1993 and April 2012 as detailed previously [4] and a call for data was issued [30]. Additional data was obtained through correspondence with authors of potentially eligible studies.

Study Selection

The study inclusion criteria was as detailed previously [4] with the following additional criteria: (1) at least 5 patients with GN bacteremia, and (2) survival outcome available on an individual patient basis in relation to endotoxemia detection and type of GN bacteremia. Additional information was received by personal communication for 11 studies.

The studies were classified as to whether they were not restricted to examining specific GN bacteremias (unrestricted studies),

versus studies that were so restricted (restricted studies) to a single GN pathogen type (e.g., *Salmonella typhi*, *Neisseria meningitidis*, *Yersinia pestis*, and *Burkholderia pseudomallei*). For the unrestricted studies, the individual patients with mono-microbial GN bacteremias were classified into four groups as follows: (1) GN bacteremias with *E. coli*, (2) GN bacteremias with Enterobacteriaceae other than *E. coli* (e.g., *Klebsiella pneumoniae*, *Enterobacter* species, *Serratia marcescens*, *Proteus mirabilis*, and *Providencia rettgeri*), (3) GN bacteremias other than Enterobacteriaceae (e.g., *Pseudomonas* species, *Acinetobacter* species, and *Bacteroides* species). According to the classification by Munford [1], the GN bacteria of the first two groups are known to have a hexa-acyl lipid A whereas those of the third group usually have a lipid A that is either non-hexa-acyl or of unknown structure. The following GN bacteremias were not included in this analysis due to small numbers and uncertainties in classification: *Campylobacter* species, *Neisseria gonorrhoeae*, non-*typhi* *Salmonellae*, *Haemophilus influenzae*.

Data Extraction and Analysis: Calculation of an Odds Ratio

The endotoxemia detection and mortality outcome data for all studies was extracted for those with GN bacteremia on a per patient basis. The determination of endotoxemia present versus absent was as recorded in the original publication without any attempt to standardize endotoxemia detection levels across publications. An odds ratio (OR) was then calculated as follows:

$$OR = (\text{endotoxemia positive with fatal outcome} / \text{endotoxemia positive with non-fatal outcome}) / (\text{endotoxemia negative with fatal outcome} / \text{endotoxemia negative with non-fatal outcome}).$$

For the purposes of graphical representation, a forest plot was generated using summary OR and 95% confidence intervals (CI) as derived using The Mantel-Haenszel method in which 0.5 was added to any cell containing a zero to avoid deriving ORs that were undefined. These calculations and graphics were undertaken using the ‘Metan’ command [31] in STATA (release 11.0; STATA Corp., College Station, Tex., USA). This enables both a visual and statistical evaluation of homogeneity [32].

There exists within the dataset the following data issues: small samples, sparse data and zero cells. Hence, two different methods of statistical analysis were undertaken. First, to allow for these issues summary level ORs and 95% CIs for each category of GN bacteremia were derived by exact grouped logistic regression (ELR) methods using the ‘exlogistic’ command in STATA.

Second, because the mortality proportions had been derived from clusters of patients within studies, the independence of the data is not a tenable assumption. This assumption is vitiated by the potentially infectious nature of some of the GN bacteremias under study. Hence, for the purpose of comparisons across categories of GN bacteremias and to derive a test for interaction between the detection of endotoxemia and the type of GN bacteremia, the method of generalized estimating equations (GEE) was used [33].

Results

There were 17 studies that were not restricted (table 1) and 10 studies that were restricted (table 2) to a single GN pathogen type.

Table 1. Mortality proportions for patients with GN bacteremia (unrestricted studies)

Authors, year [reference]	Patient population	Assay ^{a, b} ng/ml	Census ^c days	Number of patients with fatal outcome/number tested					
				<i>E. coli</i>		non- <i>E. coli</i> Entero- bacteriaceae ^d		non-Entero- bacteriaceae ^e	
				etx+	etx–	etx+	etx–	etx+	etx–
<i>Studies in an ICU setting</i>									
Bates et al., 1998 [14] ^f	Sepsis syndrome	0.005	28	0/3	6/20	4/6	4/14	1/2	2/5
Danner et al., 1991 [15] ^f	Clinically defined septic shock	0.01	14	1/5	0/4	1/2	0/3	1/2	
Dofferhoff et al., 1992 [34]	Clinically defined severe sepsis	0.005	21	0/1	0/1	1/1	0/1	1/2	
Goldie et al., 1995 [16] ^{f, g}	Sepsis syndrome	0.002	30	0/1	1/1	2/3	1/1	1/2	0/1
Guidet et al., 1994 [17] ^{f, h}	Sepsis syndrome	0.005	28	3/9	3/7	4/6		0/1	1/1
Maury et al., 1998 [35] ^{f, i}	Severe sepsis and bacteremic	0.005	30	0/8		2/4		2/3	
Strutz et al., 1999 [36]	Sepsis syndrome, ICU	0.01	NS	1/4	3/4	0/1			0/1
Opal et al., 1999 [12] (low; 20–660 pg/ml) ^{f, j}	Sepsis syndrome	0.02	28	2/15		2/12		4/8	
Opal et al., 1999 [12] (high; >660 pg/ml) ^{f, j}	Sepsis syndrome	0.66	28	5/18	2/6	4/15	1/6	7/16	3/7
	Subtotal			12/64	15/43	20/50	6/25	17/36	6/15
<i>Studies in an unrestricted setting</i>									
Martinez et al., 1973 [37] ^k	Suspected serious infection	5	NS	1/1	2/4		0/3	0/1	2/2
Byl et al., 2001 [38] ^{f, k}	Suspected sepsis	0.05 (EU/ml)	NS	2/6	0/2	0/1		0/1	0/2
Giamarellou et al., 1999 [39] ^{f, k}	Acute pyelonephritis	0.1	NS	0/2	0/8	1/1	0/1		
Prins et al., 1995 [40] ^{f, k}	Urosepsis	0.05 (EU/ml)	3	0/4	0/5			0/1	
<i>Studies in an oncology setting</i>									
Hynninen et al., 1995 [41] ^k	Febrile and neutropenic	0.013	14	0/1		0/2			
Engvall et al., 1997 [42] ^{f, k}	Febrile and neutopenic	5	NS	0/1			0/1	1/1	0/2
Yoshida et al., 1994 [43] ^{f, k}	Febrile and neutopenic	0.003	NS	1/1		4/6	1/5	3/8	0/4
<i>Studies in a pediatric setting</i>									
Shenep et al., 1988 [44] ^k	Pediatric	0.025	5		0/1	1/2		0/1	
Ahmed et al., 2004 [45] ^k	Pediatric	0.004	15					1/4	
	Subtotal			5/17	2/20	6/12	1/10	5/17	2/10
	Total			17/81	17/63	26/62	7/35	22/53	8/25

etx = Endotoxemia; NS = not stated.

^a Refers to endotoxin assay breakpoint for endotoxemia detection. ^b The endotoxemia determination was the initial [12, 14, 36, 39, 40, 42, 44, 45], median [16, 17, 34, 35], or peak [15, 37, 38] level of multiple levels or was the level taken on a single [41, 43] occasion. ^c Refers to survival census day.

^d Non-*E. coli* Enterobacteriaceae includes the following bacteremias: *Klebsiella* species, *Enterobacter* species, *Proteus* species, and *Serratia* species.

^e Non-Enterobacteriaceae includes the following bacteremias: *Pseudomonas* species, *Acinetobacter* species, and *Bacteroides* species. ^f Data for these

studies [12, 14–17, 39, 40, 42, 43] provided by personal communication.

^g Goldie et al. [16] – *H. influenzae* bacteremia (n = 1) from this study not included. ^h Guidet et al. [17] – *H. influenzae* bacteremia (n = 1) from this study not included. ⁱ Maury et al. [35] – *H. influenzae* bacteremia (n = 1) from this study not included. ^j Opal et al. [12] – data for this study stratified into two categories of endotoxemia detected: low (20–660 pg/ml) and high (>660 pg/ml) versus endotoxemia not detected (<20 pg/ml). ^k Data for these studies were collapsed into a single category to enable their representation within figure 1.

Unrestricted Studies

Eight unrestricted studies [12, 14–17, 34–36] undertaken in an ICU setting were identified reporting the results for a total of 233 GN bacteremic patients of which 76 (33%) had a fatal outcome (table 1). In these studies *E. coli* was the most common GN bacteremia and had the least mortality (107 with 27 deaths; 25%), versus non-*E. coli* Enterobacteriaceae (75 with 26 deaths; 35%) and ver-

sus non-Enterobacteriaceae (51 with 23 deaths; 45%). There were an additional 9 non-ICU studies in which there were 86 GN bacteremias in the three categories with 21 fatalities (24%). Among these 9 unrestricted non-ICU studies are 4 studies limited to adult patients [37–40], 3 studies of febrile oncology patients [41–43] and 2 studies limited to a pediatric age group [44, 45] (table 1). In only 3 studies [38–40] was it explicitly stated that endotoxemia

Table 2. Mortality proportions for patients with GN bacteremia (restricted studies)

Authors, year [reference]	Assay ^{a, b} ng/ml	Census ^c days	Number of patients with fatal outcome/number tested	
			etx+	etx–
<i>Neisseria meningitidis</i>				
Brandtzaeg et al., 1989 [9] ^d	0.005	5	9/24	0/11
Brandtzaeg et al., 1996 [46] ^d	0.005	NS	21/40	0/19
Prins et al., 1998 [47] ^d	0.05 (EU/ml)	NS	5/30	0/1
Gardlund et al., 1995 [48]	0.01	NS	1/9	0/1
Others (Goldie [16], Guidet [17], Shenep [44]) ^e			1/3	ND
<i>Yersinia pestis</i>				
Butler et al., 1973 [7]	0.5	7	1/2	ND
Butler et al., 1976 [8]	5	NS	0/3	0/2
<i>Salmonella typhi</i>				
Adinolfi et al., 1987 [49]	0.3	20	0/7	0/7
Magliulo et al., 1976 [50]	1.0	7	0/8	0/4
Suyasa et al., 1995 [51]	0.01	NS	1/4	1/6
<i>Burkholderia pseudomallei</i>				
Simpson et al., 2000 [52] ^d	0.025	28	9/15	1/5

etx = Endotoxemia; ND = no data; NS = not stated.

^a Refers to endotoxin assay breakpoint for endotoxemia detection. ^b The endotoxemia determination was the initial [7–9, 46–49, 52] level of multiple levels or was the level taken on a single

[50, 51] occasion. ^c Refers to survival census day. ^d Data for these studies [9, 46, 47, 52] provided by personal communication. ^e One meningococcemia from each of these studies [23, 24, 43].

determinations were obtained prior to the administration of antibiotic therapy whereas for the remaining studies this was not stated.

The summary ORs for the three categories of GN bacteremias are summarized in table 3 and presented as a forest plot in figure 1. The largest study [12] provided data in relation to endotoxemia detection at two breakpoints which have been used here to stratify the mortality proportions from this study within figure 1. None of the study level ORs (fig. 1) were significantly elevated above 1 for any of the three categories of GN bacteremia (table 3). The I^2 associated with each of these summary ORs was <10%, indicating minimal heterogeneity (fig. 1).

For an analysis with all the unrestricted studies included, only in the case of non-*E. coli* Enterobacteriaceae was the summary OR significantly elevated above 1, indicative of a worse outcome in endotoxemic patients (table 3). The I^2 associated with each of these summary ORs was again <10% (data not shown). The increased mortality for this category is particularly apparent for *Klebsiella* species (9 of 13 deaths in 8 studies if endotoxemia was detected vs. 2 of 17 deaths in 6 studies if endotoxemia was not detected). The method used to derive the summary ORs in either figure 1 or table 3 made no substantive difference

to the overall findings. For an analysis with only the ICU studies included, none of the summary ORs were significantly elevated above 1.

Enterobacteriaceae Bacteremias and Endotoxemia

The effect of the detection of endotoxemia on mortality for GN bacteremias with *E. coli* versus Enterobacteriaceae other than *E. coli* (table 3) was tested as an interaction term within a regression model. This interaction term was found to be significant indicating an impact of endotoxemia detection on prognosis additional to the impact of GN bacteremia type. This interaction term was found to be significant (OR 2.8; 1.01–8.1) for all unrestricted studies (table 3) and also for an analysis limited to the studies undertaken in ICU setting (OR 3.8; 1.13–12.8).

Restricted Studies

There were 10 studies that were restricted to one of the following four GN pathogen types (table 2): *Neisseria meningitidis* (4 studies [9, 46–48]), *Burkholderia pseudomallei* (1 study [52]), *Yersinia pestis* (2 studies [7, 8]), and *Salmonella typhi* (3 studies [49–51]) in which 201 bacteremic patients experienced 50 (25%) fatal outcomes. For these GN bacteremias, only in the case of *Neisseria men-*

Table 3. Summary mortality proportions and ORs in association with endotoxemia detection

GN bacteremia species	Number of studies	Dead/total number of patients (%)		OR; 95% CI	
		endotoxemia detected	endotoxemia not detected	GEE	ELR
<i>ICU studies only</i>					
<i>E. coli</i> ^a	8	12/64 (19)	15/43 (35)	0.54; 0.25–1.13	0.51; 0.17–1.5
Non- <i>E. coli</i> Enterobacteriaceae ^{a, b}	8	20/50 (40)	6/25 (24)	2.7; 0.90–8.4	3.1; 0.82–13.7
Non-Enterobacteriaceae ^c	7	17/36 (47)	6/15 (40)	1.2; 0.35–4.1	1.06; 0.27–4.3
<i>All studies</i>					
<i>E. coli</i> ^a	15	17/81 (21)	17/63 (27)	0.78; 0.36–1.7	0.81; 0.28–2.3
Non- <i>E. coli</i> Enterobacteriaceae ^{a, b}	14	26/62 (42)	7/35 (20)	3.7; 1.3–10.3	3.9; 1.1–16.7
Non-Enterobacteriaceae ^c	13	22/53 (42)	8/25 (32)	1.7; 0.65–4.6	1.4; 0.42–5.3
<i>Neisseria meningitides</i>	4	37/106 (35)	0/32 (0)	26.0; 1.6–321	36.9; 7.7–inf.
<i>Yersinia pestis</i>	2	1/5 (20)	0/2 (0)	undefined	undefined
<i>Salmonella typhi</i>	3	1/19 (5)	1/17 (6)	undefined	0.89; 0.01–74.1
<i>Burkholderia pseudomallei</i>	1	9/15 (60)	1/5 (20)	undefined	undefined

GEE = Generalized estimating equation method; ELR = exact logistic regression method.

^a The impact of the detection of endotoxemia on the difference in mortality proportions for *E. coli* versus non-*E. coli* Enterobacteriaceae bacteremias was tested as an interaction term within a regression model using generalized estimating equations. This interaction term was found to be significant for an analysis in-

cluding all studies (OR 2.8; 1.01–8.1) and for an analysis limited to the studies undertaken in ICU only (OR 3.8; 1.13–12.8).

^b Non-*E. coli* Enterobacteriaceae includes the following bacteremias: *Klebsiella* species, *Enterobacter* species, *Proteus* species, and *Serratia* species. ^c Non-Enterobacteriaceae includes the following bacteremias: *Pseudomonas* species, *Acinetobacter* species, and *Bacteroides* species.

ingitidis bacteremia was the summary OR for mortality significantly elevated (26.0; 1.6–321; $p < 0.0001$) in association with the detection of endotoxemia (table 3).

Discussion

GN bacteremia has a high mortality and observations derived from animal models implicate a key role for endotoxemia in its pathogenesis. From this, it could be hypothesized that the differences in relative frequency of detection of concomitant endotoxemia with GN bacteremia on the one hand [30, 53, 54], or the known differences in the structure activity relationship of the lipid A component of the endotoxin molecule on the other [1, 22, 27–28], might account for differences in mortality between bacteremias with Enterobacteriaceae versus other GN bacteria. However, the findings here were unexpectedly otherwise.

This analysis updates two previous meta-analyses [21, 53] applying current analytic methodology [31, 33], using available patient level data to enable questions relating to the interaction between GN bacteremia species type and endotoxemia detection on prognosis to be addressed.

There are several obstacles to resolve in undertaking the analysis presented here. Firstly, the studies were diverse with respect to their dates of publications, sizes, study designs, endotoxemia breakpoint levels, survival census times, patient populations, and settings. By contrast, the studies conducted in the ICU setting exhibited less diversity with respect to these features and were all large and published in the 1990s. Second, the data is sparse, unbalanced and clustered. In undertaking a meta-analysis the Mantel-Haenszel method is commonly used for data analysis and also to provide a graphical summary together with computational tests for heterogeneity. However, this method may be suboptimal for deriving and comparing summaries under the data conditions found here. So two additional analytic methods were employed here. Logistic regression using exact methods and the method of generalized estimating equations are more computationally intensive methods which allow a population-averaged summation under less restrictive assumptions regarding the underlying population distributions and data independence [33].

There are five findings here:

(1) The prognostic value of endotoxemia detection in association with GN bacteremia studies varies across dif-

Fig. 1. Forest plot of mortality OR of patients with GN bacteremia in relation to whether endotoxemia was (etx+) or was not (etx-) detected presented as study specific and summary OR and 95% CI. 'etx- less survival' and 'etx+ less survival' indicates the respective OR ranges for which mortality was more common in association with either endotoxemia non-detection or detection, respectively. Arrowheads indicate 95% CIs that extend out of range.

ferent GN bacteremia species. This variability across species types, which is in contrast to the lack of heterogeneity across studies, is visually apparent in the forest plot (fig. 1).

- (2) The endotoxemia detection in association with bacteremias with *Neisseria meningitidis* and with Enterobacteriaceae other than *E. coli* has a strong prognostic value.
- (3) Surprisingly, the detection of endotoxemia in association with *E. coli* bacteremia, which is the second largest GN bacteremia category here, has no prognostic value. No single study included here would have been large enough to demonstrate this observation. As a sensitiv-

ity analysis, it was found that it would require at least three studies each having at least 40 patients with *E. coli* bacteremias with an OR for mortality of >10 in association with the detection versus non-detection of endotoxemia to change the conclusions found here (data not shown). It is unlikely that such studies would be unpublished or easily overlooked.

- (4) The overall better prognosis associated with *E. coli* bacteremias [25] versus non-*E. coli* Enterobacteriaceae (table 3) cannot be attributed to endotoxemia being less frequently detectable among *E. coli* bacteremias.

(5) For the largest study [12], the 135 GN bacteremic patients were able to be classified into subgroups with high (>660 pg/ml), low (25–660 pg/ml) and non-detectable (<25 pg/ml) levels of endotoxemia. This simple dose-response analysis did not suggest a higher mortality OR with higher levels of endotoxemia in any of the three GN bacteremia categories studied (fig. 1).

These findings are particularly surprising given that the lipid A structure of LPS (endotoxin) of Enterobacteriaceae, including *E. coli*, is typically hexa-acyl, a structure which is known to have a key role toward recognition by the MD2:TLR4 receptor and the stimulation of cytokine release [1, 55].

Limitations

There were small numbers of bacteremias with *Yersinia pestis* (plague), and *Salmonella typhi* (typhoid) and the non-Enterobacteriaceae, and within these bacteremia groups there were small numbers with endotoxemia not detected. Hence the estimates of mortality proportions for these categories of GN bacteremias and the impact of endotoxemia detection are least stable to the appearance of additional data.

Many relevant patient-specific details such as age and patient comorbidities were not available. Both endotoxemia and GN bacteremia are episodic phenomena [56] and endotoxemia levels may be increased by antibiotic therapy [57]. For all but three studies the timing of antibiotic administration in relation to these determinations is unclear. Moreover, the breakpoints used to determine a positive endotoxemia result differed across the studies.

The studies examined in this analysis differed from each other in several respects and several study level factors would have potentially varied over the time period over which these studies had been conducted. For example, the antibiotic and supportive therapies, and the blood culture methods for detecting GN bacteremias, have not been considered here. These are all factors that could be expected to have varied over the more than 25 years covered in this overview, particularly with respect to the studies published prior to 1990.

Despite the diverse mix of study designs and settings, all summary ORs are associated with I^2 less than 10%, a finding that indicates minimal heterogeneity. This is a surprising finding given the diversity of patient groups, underlying risk, and clinical settings. Minimal heterogeneity was also noted with the analysis repeated with studies undertaken in non-ICU settings included. The studies undertaken in the ICU setting were of particular interest

as published benchmarks for the mortality proportions for GN bacteremias in this patient group are available [25]. For example, the median mortality proportion among >1,000 bacteremias in ICU patients abstracted from >7 large studies is 19%, 28–30% and 32% for bacteremias with *E. coli*, Enterobacteriaceae other than *E. coli*, and *P. aeruginosa*, respectively [25] which can be used as literature-derived benchmarks. Among the three GN bacteremia categories noted in the studies included here (table 3), the mortality proportions and their relative rankings are similar to these benchmarks [25].

This overview is based on a summation of observational studies. While the mortality risk in association with the detection of endotoxemia is increased for some but not all types of GN bacteremias, this overview is unable to identify the causal mechanism for this increased risk.

An increased OR for mortality in association with the detection of endotoxemia may be due to other associations. For example, the highly significant OR for mortality in association with meningococemia may be confounded by the quantitative level of bacteremia. Brandtzaeg and colleagues [58] have found that the level of endotoxemia correlates with the level of neisserial DNA in plasma in this patient group. Likewise for other GN bacteremias, the level of bacteremia, as reflected using quantitative blood culture methods [59] or in time to blood culture positivity [60, 61], is an important determinant of prognosis.

However, the kinetics of bacteremia, endotoxemia and pathophysiology may not be concordant. For example, in one clinical study [15] of 100 patients with sepsis in an ICU setting, the cumulative percent found to have endotoxemia rose from 20 to 40% between 0 and 24 h after study entry. Moreover, there are observations in animal models of septic shock conducted under controlled conditions with quantitative measurements of endotoxemia, GN bacteremia, hemodynamic changes and duration of survival which relate to this non-concordance [22, 23]. Following intraperitoneal challenge with strains of *E. coli* which have (O6:H1:K2) or do not have (O86:H8) virulence factors for human disease, survival time was shorter and the associated hemodynamic changes were more severe after challenge with the virulent strain, as might be expected. However, there are three paradoxical observations as follows: (1) bacteremia occurred earlier and more frequently after challenge with the avirulent *E. coli* strain; (2) levels of endotoxemia were three-fold higher after challenge with the avirulent *E. coli* strain, and (3) challenge with heat-killed bacteria at a ten-fold higher dose

was associated with a reversal of the effects on survival and hemodynamic change seen with live bacterial challenge: the survival was now significantly shortened after challenge with the killed non-virulent bacteria versus the killed virulent bacteria. Despite this reversal, the levels of endotoxemia were again three-fold higher after challenge with the killed avirulent versus the killed virulent *E. coli* strain [22, 23].

The co-presence of endotoxemia and GN bacteremia may also reflect important differences in host factors. In a study of 68 patients with GN bacteremia, Young [62] found a strong association between endotoxemia and recognized adverse prognostic host factors such as leukopenia, low opsonic titers in plasma against the autologous bacteremia strain and more severe underlying disease. Also, in an animal model, it has been noted that endotoxemia impedes the clearance of bacteremia [63].

It is notable that endotoxin derived from *E. coli* is used commonly in animal and preclinical studies [64]. Moreover, future clinical investigations should consider the types and co-detection of GN bacteremia as confounding factors [65] in clinical associations of endotoxemia.

Conclusion

The impact of co-detection of endotoxemia with GN bacteremia on patient prognosis is unequal for different types of GN bacteremia, even amongst Enterobacteriaceae. This finding is surprising given the commonality of

hexa-acyl lipid A structures among the endotoxins of Enterobacteriaceae. In the light of experimental findings, it is plausible that the unexpected clinical observations here may reflect differential kinetics of endotoxemia and GN bacteremia which are consequent on the presence of different virulence factors present in different species of GN bacteria [66].

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References

- Munford RS: Sensing gram-negative bacterial lipopolysaccharides: a human disease determinant? *Infect Immun* 2008;76:454–465.
- Hurley JC: Endotoxemia: methods of detection and clinical correlates. *Clin Micro Rev* 1995;8:268–292.
- Opal S, Gluck T: Endotoxin as a drug target. *Crit Care Med* 2003;31:S57–S64.
- Hurley JC, Guidet B, Offensadt G, Maury E: Endotoxemia and mortality prediction in ICU and other settings: underlying risk and co-detection of gram negative bacteremia are confounders. *Crit Care* 2012;16:R148.
- Dyson A, Singer M: Animal models of sepsis: Why does preclinical efficacy fail to translate to the clinical setting? *Crit Care Med* 2009;37:S30–S37.
- Rittirsch D, Hoesel LM, Ward PA: The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol* 2007;81:137–143.
- Butler T, Levin J, Cu DQ, Walker RI: Bubonic plague: detection of endotoxemia with the limulus test. *Ann Intern Med* 1973;79:642–646.
- Butler T, Levin J, Linh NN, Chau DM, Adickman M, Arnold K: *Yersinia pestis* infection in Vietnam. II. Quantitative blood cultures and detection of endotoxin in the cerebrospinal fluid of patients with meningitis. *J Infect Dis* 1976;133:493–499.
- Brandtzaeg P, Kierulf P, Gaustad P, et al: Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. *J Infect Dis* 1989;159:195–204.
- van Deventer SJ, Buller HR, ten Cate JW, Sturk A, Pauw W: Endotoxaemia: an early predictor of septicemia in febrile patients. *Lancet* 1988;1:605–609.
- Levin J, Poore TE, Young NS, et al: Gram-negative sepsis: detection of endotoxemia with the limulus test. With studies of associated changes in blood coagulation, serum lipids and complement. *Ann Intern Med* 1972;76:1–7.
- Opal SM, Scannon PJ, Vincent JL, et al: Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *J Infect Dis* 1999;180:1584–1589.
- Stumacher RJ, Kovnat MJ, McCabe WR: Limitations of the usefulness of the limulus assay for endotoxin. *N Engl J Med* 1973;288:1261–1264.
- Bates DW, Parsonnet J, Ketchum PA, et al: Limulus lysate assay for detection of endotoxin in patients with sepsis syndrome. *Clin Infect Dis* 1998;27:582–591.

- 15 Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Parillo JE: Endotoxemia in human septic shock. *Chest* 1991;99:169–175.
- 16 Goldie AS, Fearon KCH, Ross JA, et al: Natural cytokine antagonists and endogenous anti-endotoxin core antibodies in sepsis syndrome. *JAMA* 1995;274:172–177.
- 17 Guidet B, Barakett V, Vassal T, Petit JC, Offenstadt G: Endotoxemia and bacteremia in patients with sepsis syndrome in the intensive care unit. *Chest* 1994;106:1194–1201.
- 18 Venet C, Zeni F, Viallon A, et al: Endotoxaemia in patients with severe sepsis or septic shock. *Intensive Care Med* 2000;26:538–544.
- 19 Kollef MH, Eisenberg PR: A rapid quantitative assay to detect circulating endotoxin can predict the development of multiorgan dysfunction. *Chest* 1997;112:173–180.
- 20 Casey LC, Balk RA, Bone RC: Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann Intern Med* 1993;119:771–778.
- 21 Hurley JC: Endotoxemia and Gram-negative bacteremia as predictors of outcome in sepsis: a meta-analysis using ROC curves. *J Endotoxin Res* 2003;9:271–279.
- 22 Danner RL, Natanson C, Elin RJ, et al: *Pseudomonas aeruginosa* compared with *Escherichia coli* produces less endotoxemia but more cardiovascular dysfunction and mortality in a canine model of septic shock. *Chest* 1990;98:1480–1487.
- 23 Hoffman WD, Danner RL, Quezado ZM, Banks SM, Elin RJ, Hosseini JM, Natanson C: Role of endotoxemia in cardiovascular dysfunction and lethality: virulent and nonvirulent *Escherichia coli* challenges in a canine model of septic shock. *Infect Immun* 1996;64:406–412.
- 24 Roberts FJ, Geere IW, Coldman A: A three-year study of positive blood cultures, with emphasis on prognosis. *Rev Infect Dis* 1991;13:34–46.
- 25 Cohen J, Cristofaro P, Carlet J, Opal S: New method of classifying infections in critically ill patients. *Crit Care Med* 2001;32:1510–1526.
- 26 McCabe WR, Jackson GG: Gram negative bacteremia. II Clinical laboratory and therapeutic observations. *Arch Intern Med* 1962;110:856–864.
- 27 Dehus O, Hartung T, Hermann C: Endotoxin evaluation of eleven lipopolysaccharides by whole blood assay does not always correlate with Limulus amoebocyte lysate assay. *J Endotoxin Res* 2006;12:171–180.
- 28 Devleeschouwer MJ, Cornil MF, Dony J: Studies on the sensitivity and specificity of the Limulus amoebocyte lysate test and rabbit pyrogen assays. *Appl Environ Microbiol* 1985;50:1509–1511.
- 29 Hurley JC: Does gram negative bacteremia occur without endotoxemia? A meta-analysis using hierarchical summary ROC curves. *Eur J Clin Microbiol Infect Dis* 2010;29:207–215.
- 30 Hurley JC: Endotoxemia and gram-negative bacteremias predictors of outcome in sepsis; a call for data. *J Endotoxin Res* 2001;7:467.
- 31 Harris RJ, Bradburn MJ, Deeks JJ, Harbord RM, Altman DG, Sterne JAC: Meta-analysis: fixed and random effects-meta-analysis. *Stata J* 2008;8:3–28.
- 32 Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–560.
- 33 Mutapi F, Roddam A: p values for pathogens: statistical inference for infectious-disease data. *Lancet Infect Dis* 2002;2:219–230.
- 34 Dofferhoff ASM, Bom VJJ, de Vries-Hospers HG, et al: Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute phase proteins during the treatment of severe sepsis in humans. *Crit Care Med* 1992;20:185–192.
- 35 Maury E, Barakett V, Blanchard H, Guitton C, Fitting C, Vassal T, Chauvin P, Bertrand Guidet B, Offenstadt G: Circulating endotoxin during initial antibiotic treatment of severe gram-negative bacteremic infections. *J Infect Dis* 1998;178:270–273.
- 36 Strutz F, Heller G, Krasemann K, Krone B, Muller GA: Relationship of antibodies to endotoxin core to mortality in medical patients with sepsis syndrome. *Intensive Care Med* 1999;25:435–444.
- 37 Martinez LA, Quintiliani R, Tilton RC: Clinical experience on the detection of endotoxemia with the limulus test. *J Infect Dis* 1973;127:102–105.
- 38 Byl B, Clevenbergh P, Kentos A, Jacobs F, Marchant A, Vincent JL, Thys JP: Ceftazidime- and imipenem-induced endotoxin release during treatment of gram-negative infections. *Eur J Clin Microbiol Infect Dis* 2001;20:804–807.
- 39 Giamarellou-Bourboulis EJ, Perdios J, Lelekis M, Eoconomou E, Tsouroulas P, Giamarellou H: Impact of cefuroxime administration on endotoxin (LPS) and tumour necrosis factor- α (TNF α) blood levels in patients suffering from acute pyelonephritis: a preliminary report. *Int J Antimicrob Agents* 1999;11:115–119.
- 40 Prins JM, van Agtmael MA, Kuijper EJ, van Deventer SJ, Speelman P: Antibiotic-induced endotoxin release in patients with gram-negative urosepsis: a double-blind study comparing imipenem and ceftazidime. *J Infect Dis* 1995;172:886–891.
- 41 Hynninen M, Valtonen M, Vaara M, Markkanen H, Kuusela P, Saxen H, Takkunen O: Plasma endotoxin and cytokine levels in neutropenic and non-neutropenic bacteremic patients. *Eur J Clin Microbiol Infect Dis* 1995;14:1039–1045.
- 42 Engvall P, Granstrom M, Andersson B, Kalin M, Bjorkholm M: Endotoxemia in febrile patients with hematological malignancies. Relationship of type of bacteremia, clinical findings and serum cytokine pattern. *Infection* 1997;25:2–7.
- 43 Yoshida M, Obayashi T, Tamura H, Tanaka S, Kawai T, Sakamoto S, Miura Y: Diagnostic and prognostic significance of plasma endotoxin determination in febrile patients with hematological malignancies. *Eur J Cancer* 1994;30A:145–147.
- 44 Shenep JL, Flynn PM, Barrett FF, Stidham GL, Westenkirchner DF: Serial quantitation of endotoxemia and bacteremia during therapy for gram-negative bacterial sepsis. *J Infect Dis* 1988;157:565–568.
- 45 Ahmed T, Azam MA, Armed N, Jamil KM, Hassan F, Ogura N, Tamura H, Yokochi T: Detection of endotoxin in sera from children hospitalized for treatment of diarrhea in Bangladesh. *J Endotoxin Res* 2004;10:223–228.
- 46 Brandtzaeg P, Hogasen K, Kierulf P, Mollnes TE: The excessive complement activation in fulminant meningococcal septicemia is predominantly caused by alternative pathway activation. *J Infect Dis* 1996;173:647–655.
- 47 Prins JM, Lauw FN, Derckx BH, Speelman P, Kuijper EJ, Dankert J, van Deventer SJ: Endotoxin release and cytokine production in acute and chronic meningococcaemia. *Clin Exp Immunol* 1998;114:215–219.
- 48 Gardlund B, Sjolin J, Nilsson A, Roll M, Wickers CJ, Wretling B: Plasma levels of cytokines in primary septic shock in humans: correlation with disease severity. *J Infect Dis* 1995;172:296–301.
- 49 Adinolfi LE, Utili R, Gaeta GB, Perna P, Ruggiero G: Presence of endotoxemia and its relationship to liver dysfunction in patients with typhoid fever. *Infection* 1987;15:359–362.
- 50 Magliulo E, Scevola D, Fumarola D, Vaccaro R, Bertotto A, Burberi S: Clinical experience in detecting endotoxemia with the limulus test in typhoid fever and other *Salmonella* infections. *Infection* 1976;4:21–24.
- 51 Suyasa IG, Reka IG, Inada K, Suda H, Kojima M, Mushiaki K, Okamoto S, Yoshida M: Plasma endotoxin in typhoid fever. *Kobe J Med Sci* 1995;41:175–186.
- 52 Simpson AJ, Opal SM, Angus BJ, Prins JM, Palardy JE, Parejo NA, Chaowagul W, White NJ: Differential antibiotic-induced endotoxin release in severe melioidosis. *J Infect Dis* 2000;181:1014–1019.
- 53 Hurley JC: Reappraisal with meta-analysis of bacteremia, endotoxemia and mortality in Gram negative sepsis. *J Clin Microbiol* 1995;33:1278–1282.
- 54 Hurley JC: The detection of endotoxemia with Gram negative bacteremia is bacterial species dependent: A meta-analysis of clinical studies. *J Clin Microbiol* 2009;47:3826–3831.
- 55 Kim HM, Park BS, Kim J-I, et al: Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist eritoran. *Cell* 2007;130:906–917.
- 56 Klein DJ, Derzko A, Foster D, Seely AJ, Brunet F, Romaschin AD, Marshall JC: Daily variation in endotoxin levels is associated with increased organ failure in critically ill patients. *Shock* 2007;28:524–529.

- 57 Hurley JC: Antibiotic induced release of endotoxin: a reappraisal. *Clin Infect Dis* 1992; 15:840–854.
- 58 Øvstebo R, Brandtzaeg P, Brusletto B, Haug KB, Lande K, Høiby EA, Kierulf P: Use of robotized DNA isolation and real-time PCR to quantify and identify close correlation between levels of *Neisseria meningitidis* DNA and lipopolysaccharides in plasma and cerebrospinal fluid from patients with systemic meningococcal disease. *J Clin Microbiol* 2004;42:2980–2987.
- 59 Kreger BE, Craven DE, Carling PC, McCabe WR: Gram-negative bacteremia. III. Reassessment of etiology, epidemiology and ecology in 612 patients. *Am J Med* 1980;68:332–343.
- 60 Peralta G, Roiz MP, Sanchez MB, Garrido JC, Ceballos B, Rodriguez-Lera MJ, Mateos F, De Benito I: Time-to-positivity in patients with *Escherichia coli* bacteraemia. *Clin Microbiol Infect* 2007;13:1077–1082.
- 61 Liao CH, Lai CC, Hsu MS, et al: Correlation between time to positivity of blood cultures with clinical presentation and outcomes in patients with *Klebsiella pneumoniae* bacteraemia: prospective cohort study. *Clin Microbiol Infect* 2009;15:1119–1125.
- 62 Young LS: Opsonizing antibodies, host factors, and the limulus assay for endotoxin. *Infect Immun* 1975;12:88–92.
- 63 Koch T, Duncker HP, Axt R, Schiefer GH, Van Ackern K, Neuhaus H: Alterations of bacterial clearance induced by endotoxin and tumor necrosis factor. *Infect Immun* 1993;61:3143–3148.
- 64 Cross AS, Opal SM, Sadoff JC, Gemski P: Choice of bacteria in animal models of sepsis. *Infect Immun* 1993;61:2741–2747.
- 65 Miller PJ, Wenzel RP: Etiologic organisms as independent predictors of death and morbidity associated with bloodstream infections. *J Infect Dis* 1987;156:471–477.
- 66 Hurley JC: Reappraisal of the role of endotoxin in the sepsis syndrome. *Lancet* 1993; 341:1133–1135.