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Endotoxemia as a Diagnostic Tool for Patients with Suspected Bacteremia Caused by Gram-Negative Organisms: a Meta-Analysis of 4 Decades of Studies

James C. Hurley,[a,b] Piotr Nowak,[c] Lars Öhrmalm,[d] Charalambos Gogos,[e] Apostolos Armaganidis,[f] Evangelos J. Giamarellos-Bourboulis[g]

Rural Health Academic Center, University of Melbourne, Melbourne,[a] and Division of Internal Medicine, Ballarat Health Services, Ballarat, Victoria,[b] Australia; Institution of Medicine Huddinge, Department of Infectious Diseases, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden[c]; Department of Medicine Solna, Unit of Infectious Diseases, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden[d]; Department of Medicine, University of Patras Medical School, Patras, Greece[e]; Second Department of Critical Care Medicine, University of Athens Medical School, Athens, Greece[f]; Fourth Department of Internal Medicine, University of Athens Medical School, Athens, Greece[g]

The clinical significance of endotoxin detection in blood has been evaluated for a broad range of patient groups in over 40 studies published over 4 decades. The influences of Gram-negative (GN) bacteremia species type and patient inclusion criteria on endotoxemia detection rates in published studies remain unclear. Studies were identified after a literature search and manual reviews of article bibliographies, together with a direct approach to authors of potentially eligible studies for data clarifications. The concordance between GN bacteremia and endotoxemia expressed as the summary diagnostic odds ratios (DORs) was derived for three GN bacteremia categories across eligible studies by using a hierarchical summary receiver operating characteristic (HSROC) method. Forty-two studies met broad inclusion criteria, with between 2 and 173 GN bacteremias in each study. Among all 42 studies, the DORs (95% confidence interval) were 3.2 (1.7 to 6.0) and 5.8 (2.4 to 13.7) in association with GN bacteremias with *Escherichia coli* and those with *Pseudomonas aeruginosa*, respectively. Among 12 studies of patients with sepsis, the proportion of endotoxemia positivity (95% confidence interval) among patients with *P. aeruginosa* bacteremia (69% [57 to 79%]; $P = 0.004$) or with *Proteus* bacteremia (76% [51 to 91%]; $P = 0.04$) was significantly higher than that among patients without GN bacteremia (49% [33 to 64%]), but this was not so for patients bacteremic with *E. coli* (57% [40 to 73%]; $P = 0.55$). Among studies of the sepsis patient group, the concordance of endotoxemia with GN bacteremia was surprisingly weak, especially for *E. coli* GN bacteremia.

The *Limulus* amebocyte lysis (LAL) assay, which utilizes extracts of blood cells (amebocytes) of the *Limulus polyphemus* horseshoe crab, is a highly sensitive and specific test available for the detection of endotoxin (lipopolysaccharide [LPS]) (1). This assay is a reliable method for the detection of infection with Gram-negative (GN) bacteria in body fluids other than blood (1). However, the clinical significance of endotoxin detection in blood as both a diagnostic and a prognostic test is unclear, despite over 100 studies published over 4 decades for a broad range of patient groups (2–45). The interpretation of the literature is confounded by a 100-fold increase in assay sensitivity (3) and substantial differences in patient inclusion criteria among the studies in the literature over this period.

Moreover, in the evaluations of endotoxemia therapies over this time period, there has been a substantial and unexplained disconnect between the results of animal models of sepsis and the results of subsequent clinical trials of the same therapies (46).

Five factors have prompted a reappraisal of this question of the clinical significance of endotoxin detection. First, new studies and new data from older studies relating to endotoxemia detection have appeared and need to be incorporated (5–7, 15, 24, 32, 37, 43, 44). Second, the relevance of recently defined structural differences in lipid A, the biologically active component of LPS of different pathogens causing GN bacteremia, for endotoxemia detection needs to be clarified (47). Third, paradoxical observations among animal models of sepsis indicate that the concordance of endotoxemia with GN bacteremia and also with outcome is expected to differ for different GN bacteremia types (48). The detection of endotoxemia is of interest in relation to ongoing efforts to develop rapid detection methods for GN bacteremia and the possible application of emerging endotoxemia therapies (46). Finally, newer statistical methods have enabled a reappraisal over a broad range of assay breakpoints (49).

The purpose here was to reappraise the literature, with particular interest in patients with documented GN bacteremia within those studies that used sepsis criteria for patient inclusion.

MATERIALS AND METHODS

Data sources. The previously undertaken search of the literature was updated to February 2014. The search strategy was detailed previously (2, 3).

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Address correspondence to James C. Hurley, jamesh@bhs.org.au.

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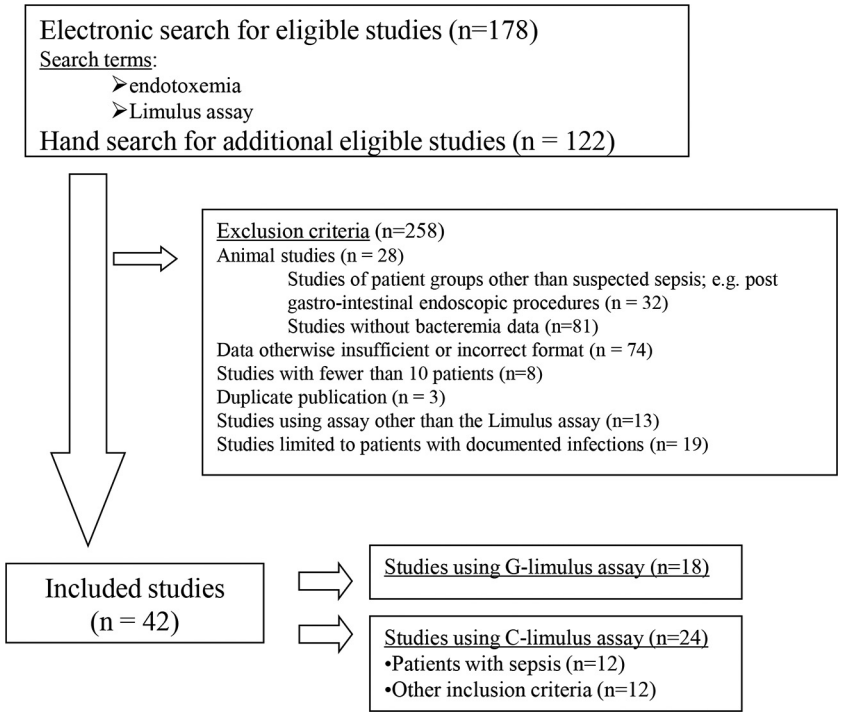

FIG 1 Flow chart of our literature search strategy and study accrual. C-limulus and G-limulus refer to the chromogenic and gelation versions of the LAL assay, respectively. Note that studies may have been excluded for more than one reason but have been counted only once.

In addition, a call for data was issued (50), and additional data were sought by personal communications with authors of potentially relevant publications.

Study selection and data extraction. The following inclusion criteria were used: (i) the study provided the results of *Limulus* assays and blood cultures for patients with suspected GN bacteremia; (ii) the study had a minimum sample size of 10 patients. The following exclusion criteria were used: animal models, studies of endotoxemia in settings other than suspected GN bacteremia (e.g., colonoscopy and intraoperative settings), studies using assays other than the *Limulus* assay, studies that were restricted to specific types of GN bacteremia (e.g., those caused by *Neisseria meningitidis*), studies which lack specific data for bacteremia or other data, and duplicate studies. Note that some studies may have been excluded for more than one reason. A complete catalog of studies excluded from the analysis was reported previously (2).

The endotoxemia detection data were extracted from each study on a per-patient basis as follows. Patients in each of the following three categories of bacteremia were counted: (i) *Escherichia coli*, (ii) *Pseudomonas aeruginosa*, (iii) non-*E. coli Enterobacteriaceae*. The last category included *Klebsiella* species, *Enterobacter* species, *Proteus* species, and *Serratia* species. Any patients with Gram-positive bacteremia or fungemia were counted in the "GN bacteremia absent" category. Polymicrobial bacteremias were not included in the analysis. For each study, a breakpoint between endotoxemia positive and negative was determined which, unless otherwise indicated, was usually the sensitivity limit for the internal endotoxin standard of the *Limulus* assay used in each study. This breakpoint was converted to the units nanograms per milliliters by using the conversion factor of 1 endotoxin unit (EU) = 0.1 ng of endotoxin, where necessary. Those patients with GN bacteremia with endotoxemia detected above versus below the breakpoint were counted as a true positive (TP), versus false negative (FN), respectively. Those patients in the category of GN bacteremia absent above versus below the breakpoint were counted as false positives (FP), versus true negatives (TN), respectively. Two studies (24, 32) provided endotoxemia data that were stratified for two breakpoints; each of these studies was analyzed by entering all study data separately for each breakpoint at half the study weight. For each study, the diagnostic odds ratio (DOR) was calculated as follows: DOR = (TP/FN)/(FP/TN).

Data analysis. The derivations of the summary statistics for the DOR, sensitivity, and specificity were performed using the metandi command in STATA (release 10.0; STATA Corp., College Station, TX, USA) as previously described (2, 3). This command fits a two-level mixed logistic regression model, with independent binomial distributions for the true positives and true negatives conditional on the sensitivity and specificity in each study and a bivariate normal model for the logit transforms of sensitivity and specificity between studies. The metandi command also generates a plot containing the following: a hierarchical summary receiver operating characteristic (HSROC) curve derived from the individual study results, which were represented as data points proportional to study size, and the sensitivity and specificity, conjointly summarized as a single summary point surrounded by 95% confidence and 95% prediction ellipses.

RESULTS

This analysis included 42 studies published between the years 1970 and 2013 (4–45) (Fig. 1; Table 1). There were 8 studies with data clarifications obtained through personal communication, 10 studies not included in a previous meta-analysis (5–7, 15, 22, 24, 32, 37, 43, 44), and 3 studies not published in English (8, 15, 43). The reported assay sensitivities to the internal endotoxin standard in the studies varied by >100-fold and typically ranged between 0.1 and 10 ng/ml for the 18 studies that used the gelation version of the LAL assay, compared to a range between 0.001 and 0.1 ng/ml for the 24 studies that used the chromogenic version of the LAL assay. There were 21 studies published up to or including 1990 and 21 that were published after 1990. All but one of the studies pub-

TABLE 1 Studies analyzed to determine the concordance of endotoxemia with Gram-negative bacteremia

First author, yr (reference)	LAL version[a]	Sensitivity limit[b] (ng/ml)	Patient population	Total no. of patients
Ahmed, 2004 (4)	C	0.004	Pediatric	35
Bailey, 1976 (5)	G	5	Surgical	24
Bion, 1994 (6)	C	0.02	Surgical	52
Byl, 2001 (7)	C	0.005*	Sepsis syndrome	23
Clumeck, 1977 (8)	G	3	Unrestricted	46
Cooperstock, 1985 (9)	G	1	Pediatric	37
Danner, 1991 (10)[c]	C	0.01	Sepsis syndrome	96
Dofferhoff, 1992 (11)	C	0.005	Sepsis syndrome	18
Engervall, 1997 (12)	C	0.005*	Neutropenic	22
Feldman, 1974 (13)	G	1	Pediatric	78
Fossard, 1974 (14)	G	1	Surgical	25
Garcia Curiel, 1979 (15)	G	1	Shock	41
Giamarellos-Bourboulis, 1999 (16)	C	0.1	Urosepsis	25
Goldie, 1995 (17)[c]	C	0.002	Sepsis syndrome	129
Guidet, 1994 (18)[c]	C	0.005	Sepsis syndrome	81
Hass, 1986 (19)	C	0.01	Pediatric	35
Hynninen, 1995 (20)	C	0.013	Neutropenic	98
Jirillo, 1975 (21)	G	1	Pediatric	10
Kelsey, 1982 (22)	G	50	Pediatric	30
Ketchum, 1997 (23)[c]	C	0.005	Sepsis syndrome	362
Kritselis, 2013 (24)[c,d]	C	0.6	Sepsis syndrome	341
Kritselis, 2013 (24)[c,d]	C	0.025	Sepsis syndrome	341
Lau, 1996 (25)	C	0.01	Surgical	38
Levin, 1970 (26)	G	5	Unrestricted	93
Levin, 1972 (27)	G	5	Suspected bacteremia	217
Martinez, 1973 (28)	G	5	Suspected bacteremia	75
Massignon, 1996 (29)[b]	C	0.004	Sepsis syndrome	55
McCartney, 1987 (30)	C	NS	Neutropenic	26
Oberle, 1974 (31)	G	0.5	Pediatric	23
Opal (high), 1999 (32)[c,d]	C	0.6	Sepsis syndrome	727
Opal (low), 1999 (32)[c,d]	C	0.02	Sepsis syndrome	727
Pearson, 1985 (33)	G	0.1	Unrestricted	41
Prins, 1995 (34)[c]	C	0.04	Urosepsis	30
Scheifele, 1985 (35)	G	0.2	Pediatric	43
Shenep, 1988 (36)	G	0.025	Pediatric	20
Strutz, 1999 (37)	C	0.01	Sepsis	28
Stumacher, 1973 (38)	G	0.5	Unrestricted	126
Suyasa, 1995 (39)	G	0.01	Unrestricted	13
Togari, 1983 (40)	G	0.5	Pediatric	10
Van Deventer, 1988 (41)	C	0.005	Unrestricted	433
Van Dissel, 1993 (42)	C	NS	Sepsis syndrome	14
Watzke, 1987 (43)	C	0.01	Unrestricted	20
Wong, 2013 (44)	C	0.003	Neutropenic	103
Yoshida, 1993 (45)[c]	C	0.003	Neutropenic	125

[a] LAL assay version abbreviations: C, chromogenic; G, gelation.
[b] The *Limulus* assay sensitivity limit to an internal control endotoxin standard (in ng/ml). For those studies which used EU rather than ng/ml (marked with an asterisk), a conversion based on the formula 1 EU = 100 pg was used. NS, not specified.
[c] Data provided via personal communication with the study author.
[d] For two studies (24, 32), there is a double entry of data in the table because patients were analyzed at both high and low endotoxemia detection breakpoints. In the HSROC analysis (Table 3; Fig. 2 and 3), the study weights for the data for each breakpoint from these two studies were halved.

lished after 1990 used the chromogenic version of the LAL assay (Table 1).

There were two studies (24, 32) for which the patients were classified at two breakpoints into subgroups with either high (>660 pg/ml), low (25 to 660 pg/ml), or nondetectable (<25 pg/ml) levels of endotoxemia. There were 12 studies (14 groups) that were limited to patients with either sepsis or septic shock. All of these 12 studies used the chromogenic version of the LAL assay, and 11 of these 12 studies were published after 1990. The other 30 studies examined a diverse range of patient groups, such as pediatric, perioperative, febrile neutropenic oncology, or otherwise-unspecified adult patient groups, and used either version of the LAL assay.

The 42 studies included data for 3,868 patients, with 1,389 patients among the 10 newly included studies (5–7, 15, 22, 24, 32, 37, 43, 44). The number of patients in each study with GN bacteremia was lower among studies published up to and in 1990 (pre-1990, median of 9 patients, with an interquartile range of 3 to 16) versus those published after 1990 (post-1990, median of 15 patients with an interquartile range of 8 to 21). There were 295 *E. coli* (Fig. 2), 239 non-*E. coli Enterobacteriaceae*, and 133 *P. aeruginosa* (Fig. 3) GN bacteremias (Table 2). The concordance between the detection of endotoxemia and GN bacteremia is displayed in the HSROC plots (Fig. 2 and 3), and the summary DORs are shown in Table 3. The DORs were lower among the 12 studies limited to patients with sepsis than were the DORs among all studies.

Among the 12 studies limited to patients with sepsis, the proportion of patients without GN bacteremia who were endotoxemia positive was 49% (95% confidence interval [CI], 33 to 64%). In contrast, the proportions of patients with detectable endotoxemia among bacteremic patients for these 12 studies were as follows: *E. coli* bacteremias, 57% (CI, 40 to 73%; 12 studies); *Klebsiella* bacteremias, 41% (CI, 29 to 55%; 9 studies); *Enterobacter* bacteremias, 32% (CI, 10 to 67%; 5 studies); *Proteus* bacteremias, 76% (CI, 51 to 91%; 3 studies); *Serratia* bacteremias, 32% (CI, 10 to 67%; 1 study); *P. aeruginosa* bacteremias, 69% (CI, 57 to 79%; 9 studies). Only in the cases of patients with bacteremia caused by *P. aeruginosa* ($P = 0.004$) or *Proteus* species ($P = 0.04$), and not those with bacteremia caused by *E. coli* ($P = 0.55$) or other *Enterobacteriaceae*, were the differences compared to those in patients without GN bacteremia statistically significant. Among the 12 studies limited to patients with sepsis, the DORs were marginal, and the DORs in relation to *E. coli* bacteremias and the category of non-*E. coli Enterobacteriaceae* bacteremias, but not that in relation to *P. aeruginosa*, included unity within the respective 95% confidence intervals (Table 3).

DISCUSSION

Endotoxin is present in all GN bacteria. Hence, it might be expected that endotoxemia detection, especially in newer assays, would perform better as a diagnostic marker of GN bacteremia than methods using clinical criteria, which perform poorly (51–53). Moreover, GN bacteremia has a high mortality in association with sepsis despite antibiotic therapy, and observations derived from animal models generally, but not always (48, 54), have implicated a key role for endotoxemia in GN bacteremia pathogenesis. Hence, it might also be anticipated that the concordance between endotoxemia and GN bacteremia would be high in those patients with sepsis. However, the diagnostic and prognostic significance of endotoxemia is complex, and the findings here are somewhat at variance with these expectations.

Endotoxemia is detected in approximately half of those with GN bacteremia, and similarly, GN bacteremia is detected in ap-

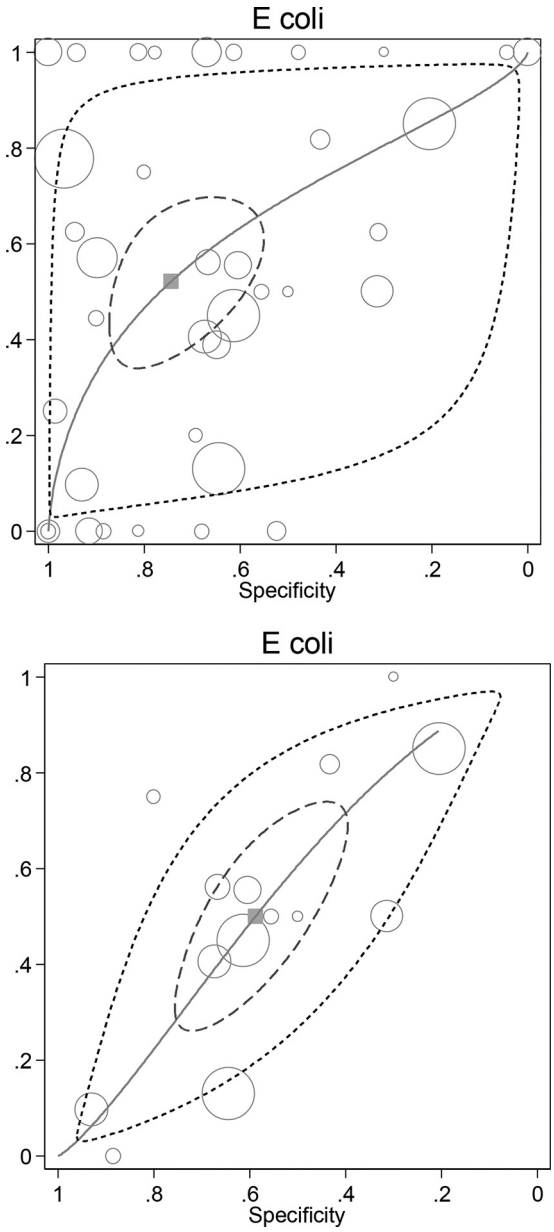

FIG 2 Plot of sensitivity versus specificity from 37 studies for all patients populations (39 groups; top plot) and for patients with sepsis (14 groups; bottom plot) for the detection of endotoxemia using the *Limulus* assay versus *E. coli* bacteremia, together with the fitted HSROC curve and the bivariate summary estimate (solid square) for sensitivity and specificity together with the corresponding 95% confidence ellipse (inner broken line) and 95% prediction ellipse (outer dotted line). The symbol size for each study is proportional to the study size.

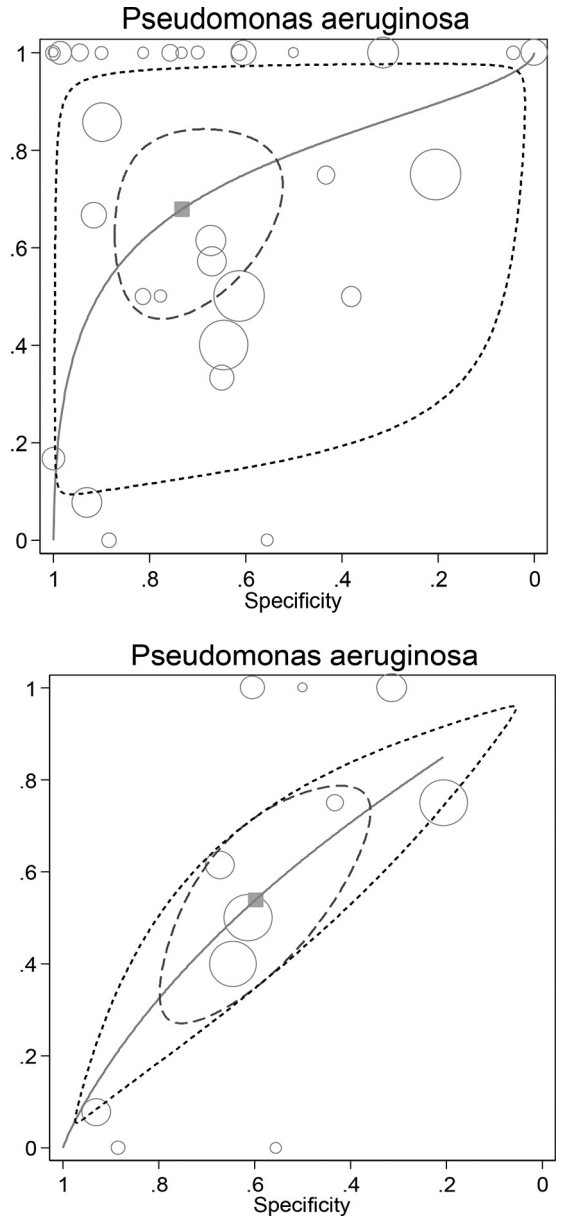

FIG 3 Plot of sensitivity versus specificity for 31 studies for all patient populations (33 groups; top plot) and for patients with sepsis (11 groups; bottom plot) for the detection of endotoxemia using the *Limulus* assay versus *Pseudomonas aeruginosa* GN bacteremia together with the fitted HSROC curve and the bivariate summary estimate (solid square) for sensitivity and specificity, together with the corresponding 95% confidence ellipse (inner broken line) and 95% prediction ellipse (outer dotted line). The symbol size for each study is proportional to the study size.

proximately half of those with endotoxemia. It has been established in previous analyses that the concordance of endotoxemia with GN bacteremia overall is weak regardless of whether a more- or less-sensitive version of the LAL assay is used (3). The questions addressed here were the additional influences of GN bacteremia species type and the use of sepsis criteria for patient inclusion on this concordance and on the endotoxemia detection rates in studies published over the past 4 decades.

There were wide ranges of GN bacteremia types, patient groups, and study sizes among the studies published since the original studies of the *Limulus* assay for endotoxemia detection in the early 1970s. The findings here indicate that the concordance of endotoxemia with GN bacteremias differs between GN bacteremias of different types. The concordance is surprisingly weak, especially for *Enterobacteriaceae* GN bacteremia and among studies of patients with sepsis. The concordance between endotoxemia and GN bacteremia found here was higher for *P. aeruginosa* than for most *Enterobacteriaceae*. Among stud-

TABLE 2 Detection rates by species group for each of the studies included in our analysis

First author, yr (reference)	Detection rate (no. of patients) among species group[a]							
	E. coli		Non-*E. coli* *Enterobacteriaceae*		*P. aeruginosa*		Non-GN bacteremia	
	TP	FN	TP	FN	TP	FN	FP	TN
Ahmed, 2004 (4)	1	0	0	0	3	0	12	19
Bailey, 1976 (5)	1	0	0	0	0	0	12	11
Bion, 1994 (6)	0	0	0	0	1	1	31	19
Byl, 2001 (7)	6	2	0	0	0	0	3	12
Clumeck, 1977 (8)	5	3	0	0	2	0	2	34
Cooperstock, 1985 (9)	3	0	0	0	1	1	6	26
Danner, 1991 (10)[b]	5	4	1	3	2	0	32	49
Dofferhoff, 1992 (11)	1	1	1	1	2	0	6	6
Engervall, 1997 (12)	1	0	0	1	1	1	4	14
Feldman, 1974 (13)	0	9	0	4	2	10	0	53
Fossard, 1974 (14)	1	0	0	0	1	0	22	1
Garcia Curiel, 1979 (15)	0	3	1	10	0	1	3	23
Giamarellos-Bourboulis, 1999 (16)[b]	2	8	1	1	0	0	4	9
Goldie, 1995 (17)[b]	1	1	3	1	2	0	83	38
Guidet, 1994 (18)[b]	9	7	5	0	0	0	20	40
Hass, 1986 (19)	0	0	0	0	2	0	8	25
Hynninen, 1995 (20)	1	0	1	0	0	0	0	96
Jirillo, 1975 (21)	0	0	1	1	0	0	4	4
Kelsey, 1982 (22)	0	1	0	0	1	0	0	28
Ketchum, 1997 (23)[b]	3	20	5	22	2	3	109	198
Kritselis, 2013 (24)[b,c]	6	56	9	45	1	23	13	188
Kritselis, 2013 (24)[b,c]	25	37	24	30	15	9	66	135
Lau, 1996 (25)	5	3	1	0	0	0	20	9
Levin, 1970 (26)	0	2	0	5	2	1	7	76
Levin, 1972 (27)	4	3	6	10	6	1	19	168
Martinez, 1973 (28)	1	3	0	3	1	0	1	66
Massignon, 1996 (29)	9	2	3	0	3	1	21	16
McCartney, 1987 (30)	0	1	0	0	0	0	8	17
Oberle, 1974 (31)	0	0	1	0	2	0	6	14
Opal (high), 1999 (32)[b,c]	18	21	15	18	16	15	241	383
Opal (low), 1999 (32)[b,c]	33	6	27	6	24	7	496	128
Pearson, 1985 (33)	5	0	1	1	0	0	2	32
Prins, 1995 (34)[b]	4	5	0	0	1	0	2	18
Scheifele, 1985 (35)	0	1	0	0	0	0	20	22
Shenep, 1988 (36)	0	1	2	0	1	0	3	13
Strutz, 1999 (37)	4	4	1	0	0	1	8	10
Stumacher, 1973 (38)	7	11	10	18	2	4	26	48
Suyasa, 1995 (39)	0	0	0	0	4	0	0	9
Togari, 1983 (40)	0	0	1	0	0	0	7	2
Van Deventer, 1988 (41)	7	2	4	0	0	0	14	406
Van Dissel, 1993 (42)	2	0	2	0	0	0	7	3
Watzke, 1987 (43)	0	0	3	1	1	0	4	11
Wong, 2013 (44)	8	0	5	0	1	0	89	0
Yoshida, 1993 (45)[b]	1	0	6	5	4	3	35	71

[a] Abbreviations: TP, true positive, the number of patients with endotoxemia and Gram-negative bacteremia; FP, false positive, the number of patients with endotoxemia and without Gram-negative bacteremia; FN, false negative, the number of patients without endotoxemia and with Gram-negative bacteremia; TN, true negative, the number of patients with neither endotoxemia nor Gram-negative bacteremia. Patients with polymicrobial bacteremia were not included in the analysis.

[b] Data provided via personal communication with the study author.

[c] For two studies (24, 32), there is a double entry of data in the table because patients were analyzed at both high and low endotoxemia detection breakpoints. In the HSROC analysis (Table 3; Fig. 2 and 3), the study weights for the data for each breakpoint from these two studies were halved.

ies limited to patients with sepsis, only in the cases of bacteremia with either *P. aeruginosa* or *Proteus* spp. was the proportion with endotoxemia found to be significantly above the background detection rate.

Moreover, the prognostic significance of endotoxemia is dependent on the copresence of GN bacteremia and is unequal for GN bacteremias of different types. For example, endotoxemia with *E. coli* bacteremia has no prognostic significance (55). The somewhat surprising finding here is that the concordance between endotoxemia and GN bacteremia was lower within studies that used sepsis criteria for patient inclusion versus studies that selected patients more broadly, despite the fact that the studies of

TABLE 3 Summary data for endotoxemia concordance with GN bacteremia

GN bacteremia species group	DOR[a] (95% CI), no. of studies	
	All studies	Studies of sepsis[b]
E. coli	3.2 (1.7–6.0), 37	1.4 (0.89–2.3), 14
Non-*E. coli Enterobacteriaceae*	2.8 (1.5–5.5), 31	1.5 (0.82–2.8), 13
Pseudomonas aeruginosa	5.8 (2.4–13.7), 31	1.7 (1.02–3.0), 11

[a] DOR = (TP/FN)/(FP/TN), derived using HSROC meta-analysis.
[b] All studies of sepsis used chromogenic versions of the LAL assay, which are typically 100-fold more sensitive than gelation-based versions.

patients with sepsis all used the more sensitive chromogenic version of the *Limulus* assay.

This finding is surprising for three reasons. In the application of any assay, the use of a more sensitive version would be expected to achieve a higher true-positive rate (sensitivity). However, this is generally achieved at the cost of a lower true-negative rate (specificity), an expected trade-off (49). Also, given the presumption of a key role of endotoxemia in the mediation of sepsis, a higher concordance between endotoxemia and GN bacteremia would have been expected among studies of patients meeting the criteria for sepsis versus studies of patients more broadly selected. The third reason relates to the complex interrelation between the pathogenesis of bacteremia on the one hand and, on the other hand, the structure-function activity related to how endotoxemia is sensed by the host immune system, which is dependent on the specific lipopolysaccharide structure, and also with regard to how it is detected in the *Limulus* assay, which is not so dependent.

In this regard, there are key structural differences between the lipid A components of the endotoxin molecule (LPS) of different GN bacteria. *Enterobacteriaceae* such as *E. coli* characteristically have a lipid A with a hexa-acyl structure, whereas other lipid A structures are present in non-*Enterobacteriaceae* species, such as *P. aeruginosa* (47). The hexa-acyl structure of lipid A is now known to be optimal for the recognition of GN bacteremia by the host immune system via the MD2–Toll-like receptor 4 interaction and the stimulation of cytokine release. The lipid A structure is not critical for sensing by the clotting proteins of the blood cells of the *Limulus polyphemus* horseshoe crab, from which the LAL assay was derived.

It should be noted that the LAL assay is an assay for endotoxin, not for GN bacteria *per se*. With an amount of LPS of ~0.025 pg/CFU from *E. coli* or *P. aeruginosa*, it would be expected that there would be 0.25 pg/ml of endotoxin for a bacteremia with 10 viable bacteria per 1 ml of blood (56). Even if this estimated total amount of bacterial cell-bound endotoxin were to be completely available for detection in association with a bacteremia, this would still be below the detection limit of even the most sensitive LAL assay (5 pg/ml). In contrast, but in line with these estimates, in experimental rabbit (57, 58) and canine (59) models of GN bacteremia with either *E. coli* or *Pasteurella* sp. bacterial challenge, GN bacteremias at levels of ~10,000 CFU/ml corresponded to endotoxemia levels of 10 ng/ml (58), 500 ng/ml (57), and 50 EU/ml (~5 ng/ml) (59). Note, however, that these bacteremia levels are approximately 1,000 times higher than those typically seen in sepsis in humans.

The quantitative relationship between endotoxemia and GN bacteremia is not simple and is subject to influence from several additional bacterial, physicochemical, and patient factors. In particular, the activity of endotoxin is not a uniform gravimetric property for endotoxins of different bacterial origins. Also, the mode of LPS aggregation (60), the interactive effect of plasma (61), and the presence of nonviable bacterial cells and cell fragments that accompany a GN bacteremia (62) influence the relationship. Moreover, differential kinetics of endotoxemia and GN bacteremia are likely each influenced by the presence of virulence factors, which differ for different species of GN bacteria (63).

There are several strengths of this analysis. A broad range of studies have been included, in order to address the impact of factors that cannot be addressed in an animal model of sepsis. The specification of the type of GN bacteremia was a requirement for inclusion in this analysis. The number of studies increased after additional data were sought from authors of potentially eligible studies to enable their inclusion. This resulted in a substantial increase in eligible studies and patient data available, compared to those included in a previous analysis (3). Also, the method of meta-analysis was optimal to enable the inclusion of studies across a broad range of assay breakpoints and studies of various sizes. No single study among those included here would have been sufficiently powered to answer the questions of interest. The findings would not have been achievable using any previously available meta-analytic method. Moreover, there were 12 studies of patient groups with sepsis, and the restricted concordance appeared consistent across this subgroup of studies; hence, the findings appear to be generalizable. The patient group with sepsis is of particular interest, as it would be in this group that any new therapies for either endotoxemia or sepsis would likely be tested.

The influence of several other study parameters, such as study size, study design quality, and method of plasma pretreatment, have been considered elsewhere (2, 3). For example, a disproportionate number of small studies ($n < 25$) had 100% sensitivity for the detection of endotoxemia (3). Otherwise, these parameters were each found to have a minor impact on concordance compared to the factors identified here.

The findings here are in contrast to paradoxical observations from a controlled model of septic shock in dogs. In the dog, implantation of an intraperitoneal infected clot induces bacteremia with various selected Gram-negative and Gram-positive challenge bacteria, together with cardiovascular changes characteristic of septic shock leading to mortality (48, 54, 59, 65, 66). This model enables the study of quantitative levels of endotoxemia as measured with the chromogenic LAL assay as well as bacteremia. In comparative studies with this model, a relatively avirulent strain of *E. coli* versus *P. aeruginosa* (48), *Staphylococcus aureus* (66), or a more virulent strain of *E. coli* (65) showed similar quantitative levels of bacteremia, whereas the associated hemodynamic changes and shortened survival times were in each case more severe than those observed in association with the avirulent *E. coli* strain, as would be expected (48, 65, 66). Surprisingly, despite these expected differences, in each study the levels of endotoxemia were 3-fold (65) to 10-fold (48) lower or even undetectable (46) versus the levels seen after challenge with the avirulent *E. coli* strain. Interestingly, endotoxins extracted and purified from the virulent and avirulent *E. coli* strains were equal with respect to potency and endotoxin amount per bacterium; hence, the lack of an association between endotoxemia and disease severity in this experimental model could not be explained on this basis (65).

Moreover, following intraperitoneal challenge with strains of *E. coli* with (O6:H1:K2) or without (O86:H8) virulence factors for

human disease, survival times were shorter and the associated hemodynamic changes were more severe after challenge with the virulent strain, as might be expected. However, there were three paradoxical observations: (i) bacteremia occurred earlier and more frequently after challenge with the avirulent *E. coli* strain; (ii) levels of endotoxemia were 3-fold higher after challenge with the avirulent *E. coli* strain; (iii) challenge with heat-killed bacteria at a 10-fold-higher dose was associated with a reversal of the effects on survival and hemodynamic changes seen with live bacterial challenge, as the survival was significantly shortened after challenge with the killed nonvirulent bacteria versus the killed virulent bacteria. Despite this reversal, the levels of endotoxemia were again 3-fold higher after challenge with the killed avirulent versus the killed virulent *E. coli* strains (65).

Limitations. There are several limitations of this analysis. Both endotoxemia and GN bacteremia are episodic phenomena, and endotoxemia levels may be increased by antibiotic therapy (64). For example, in one clinical study (10) of 100 patients with sepsis in an intensive care unit (ICU) setting, the cumulative percentage of patients found to have endotoxemia rose from 20% to 40% between 0 and 24 h after study entry. For all but three studies included here, the timing of antibiotic administration in relation to determinations of endotoxemia and bacteremia is unclear.

The studies included in our analysis were published over a period of over 40 years, during which time supportive and antibiotic therapies and underlying patient prognosis factors likely varied. Many relevant patient-specific details, such as age and patient comorbidities, were not available. The findings in this analysis differ slightly from the findings of a previous analysis (3) in which the summary DORs were higher than those derived here. This difference may be a consequence of the studies included in the present analysis being slightly larger in size and mostly more recently published, as well as our analysis having been restricted to bacteremias within three categories of GN bacterial species.

Even with data for 3,868 patients from 42 studies published over the past 4 decades, it is still unclear how substantial the differences are in the proportion of patients with detectable endotoxemia for different GN bacteremia species. The estimates here imply differences that may be as great as 40%. However, given the small numbers for each species, the associated 95% confidence intervals are as wide as 50%.

Another limitation is that the estimations of endotoxemia detection and the detection of bacteremia in the studies here do not take into account bacterial cells that were either nonviable or difficult to grow using current methods, as well as cell fragments associated with a GN bacteremia and how these associations may differ for different specific types of GN bacteremia (62, 63). For example, for *Neisseria meningitidis*, the concordance of endotoxemia is higher for these fragments than it is for viable bacterial cells (63).

Conclusion. The concordance between endotoxemia and GN bacteremia differs for different types of GN bacteremia and is marginal among studies using sepsis criteria for patient inclusion.

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