The Specific Organism: Not Bacterial Gram Type: Drives the Inflammatory Response in Septic Shock

Adam Linder^a Chris D. Fjell^b Malin Inghammar^a Joseph Hsu^c
Keith R. Walley^b John H. Boyd^b James A. Russell^b

^aDivision of Infection Medicine, Department of Clinical Sciences, Lund University, Lund, Sweden; ^bCentre for Heart Lung Innovation and Division of Critical Care Medicine, St. Paul's Hospital, University of British Columbia, Vancouver, BC, Canada; ^cPulmonary and Critical Care Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Keywords

Septic shock · Organism · Pathogen · Gram type · Inflammation · Cytokines

Abstract

Background and Hypothesis: The inflammatory response was targeted by unsuccessful therapies but ignored pathogen. We hypothesized that the inflammatory response differs according to organism in human septic shock. **Materials and Methods:** We measured 39 cytokines at baseline and 24 h in patients ($n = 363$) in the Vasopressin and Septic Shock Trial (VASST). We compared cytokine profiles (cytokine functional class) at baseline and at 24 h by organism and used hierarchical clustering to classify cytokines according to 28-day outcomes. **Results:** In 363 patients, 88 and 176 patients had at least 1 species isolated from blood and other sites, respectively. Cytokine levels differed significantly according to organism: *Neisseria meningitidis* and *Streptococcus pneumoniae* had the highest (baseline and at 24 h), while *Enterococcus faecalis* (blood) had the lowest mean cytokine levels. *N. meningitidis* and *Klebsiella pneumoniae* had significantly higher cytokine levels at baseline versus 24 h ($p = 0.01$ and 0.02 , re-

spectively); *E. faecalis* had significantly higher cytokine levels at 24 h versus baseline. Hierarchical clustering heat maps showed that pathogens elicited similar cytokine responses not related to the functional cytokine class. **Conclusion:** The organism type induces different cytokine profiles in septic shock. Specific gram-positive and gram-negative pathogens stimulated similar plasma cytokine-level patterns.

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Introduction

Septic shock is increasingly common with high, but declining short- [1] and long-term mortality rates [2]. Many different pathogens [3] cause septic shock, and organism type and site of infection may be an important determinant of outcome [4]. Recently, the prevalence of gram-positive bacteria has increased and is similar to or even higher [5] than the prevalence rate of gram-negative infections [6–8]. However, about two-thirds of patients with septic shock never have positive blood cultures and about one-third have no positive cultures from any site [9, 10].

A meta-analysis of 510 studies found that gram-negative bacteremia was associated with a significantly higher mortality than gram-positive bacteremia [11], implying that organism gram type should be incorporated into the risk stratification of randomized controlled trials (RCTs) in septic shock. However, Zahar et al. [12] found no difference in mortality rates according to organism gram type.

Several physiological responses to infection in septic shock are similar according to gram type such as the hemodynamic response [13–15], but there is no correlation between the organism gram type and the presence or severity of sepsis-associated coagulopathy [16]. Therefore, 2 assumptions were often made: first, proinflammatory mediators were of similar importance in septic shock regardless of the gram type and, second, that inhibition of proinflammatory mediators in all patients, independent of infecting organism gram type, was unlikely to cause harm.

The host inflammatory response has been targeted by many unsuccessful therapies (e.g., anti-tumor necrosis factor [TNF] [17], TNF receptor [18], interleukin [IL]-1ra [19], anti-IL-6, tissue factor pathway inhibitor [20]). However, different inflammatory response according to organism type could explain in part the lack of success of prior RCTs because prior RCTs targeted pooled patients regardless of organism. There are hints that organism type might be an important determinant and predictor of response to anti-inflammatory therapies. For example, p75 soluble TNF receptor treatment was efficacious in patients with gram-negative sepsis but possibly harmful in patients with gram-positive sepsis [18, 21], and corticosteroids, platelet-activating factor antagonists, and anti-endotoxin therapies had some benefit in gram-negative sepsis but no effect in gram-positive sepsis [22].

Prior studies of gram types of organisms and the human host cytokine response (as reflected by plasma levels) are contradictory with studies showing both differences and similarities according to organism gram type [23–25] further clouding the interpretation of prior trials in which patients were pooled regardless of organism type. We hypothesized that the inflammatory response at baseline and at 24 h differs according to organism, as opposed to gram type, in patients with septic shock.

Materials and Methods

Subjects

The Vasopressin and Septic Shock Trial (VASST) [10] was a multicenter randomized double-blind controlled trial of vasopressin plus norepinephrine versus norepinephrine alone in septic shock (Current Controlled Trials number ISRCTN94845869). Eth-

ics approval was obtained for the VASST study from the University of British Columbia/Providence Health Care Research Ethics Board. Written informed consent was obtained from all patients, their next of kin, or another surrogate decision maker. The VASST study enrolled 778 patients who were older than 16 years of age and had septic shock, defined by the presence of 2 or more systemic inflammatory response syndrome criteria [26], proven or suspected infection, new dysfunction of at least 1 organ, and hypotension despite adequate fluid resuscitation (lack of response to 500 mL normal saline) and requiring vasopressor support of at least 5 mg/min of norepinephrine (or equivalent) for 6 h. Important exclusion criteria were unstable coronary syndromes, acute mesenteric ischemia, severe chronic heart disease (New York Heart Association class III and IV), and vasospastic diathesis. Of these patients, 363 had blood plasma samples taken at baseline (enrolment at a median of 12 h following meeting septic shock inclusion criteria) and 24 h later. Since we studied the impact of pathogens on the inflammatory response, we excluded patients who had negative cultures.

Plasma Cytokine Assay

In this study, we included patients for whom we had both baseline and 24-h plasma samples. A panel of 39 cytokines were measured in duplicate by Luminex MAG 39plex multiplex bead assay on a 100/200 System (Luminex Corporation, Austin, TX, USA) according to the manufacturer's specifications. Positive and negative controls were assayed on each plate. The cytokines measured were (using both current and historical naming) CCL11 (Eotaxin), CCL2 (MCP1), CCL22 (MDC), CCL3 (MIP1a), CCL4 (MIP1B), CCL7 (MCP3), CD40LG (CD40Ligand), CSF2 (GM-CSF), CSF3 (G-CSF), CX3CL1 (Fractalkine), CXCL1 (GRO), CXCL10 (IP10), EGF (EGF), FGF2 (FGF2), FLT3LG (Flt3L), IFNA2 (IFNa2), IFNG (IFNG), IL10 (IL10), IL12B (IL12B), IL12P70 (IL12P70), IL13 (IL13), IL15 (IL15), IL17A (IL17), IL1A (IL1a), IL1B (IL1B), IL1RN (IL1RA), IL2 (IL2), IL2RA (IL2RA), IL3 (IL3), IL4 (IL4), IL5 (IL5), IL6 (IL6), IL7 (IL7), IL8 (IL8), IL9 (IL9), LTA (TNFB), TGFA (TGFA), TNF (TNFA), and VEGFA (VEGF).

Values were reported by the assay system in pg/mL; these were converted to molar concentrations using Ensembl mature peptide amino acid sequences and molecular weights calculated using *pepstats* from the EMBOSS 4.1.0 software.

Microbiology

All culture results for the 48 h prior to and after inclusion in VASST as reported by each site's clinical microbiology laboratory were recorded. Organisms were classified as gram positive or negative according to conventional definitions.

Statistical Methods

Comparison of Cytokine Levels

All statistics were calculated using the base *stats* package of the R statistical language. Cytokine levels were compared using *t* test with Welch's method for unequal variance (*t* test method). Multiple-comparison adjustment was done using false discovery rate (FDR) of Benjamini-Hochberg method, using *p. adjust* method; adjusted values are reported as p_{adj} , $p_{adj} < 0.05$ was taken as significant. Cytokine levels were log₁₀-transformed before comparison to make their distribution approximately normal. Cytokines with values of zero were assigned to 1/2 the minimum cytokine level detected overall for any cytokine to allow log-transformation.

Table 1. Demographics

Variables	All patients	Blood culture positives	Other site culture positives
<i>Number</i>	363	88	176
Age, years, mean (SD)	63 (50–72)	62 (50–72)	62 (50–72)
Gender female, <i>n</i> (%)	147 (40)	39 (44)	65 (37)
<i>Comorbidities</i>			
Cong. heart failure, <i>n</i> (%)	25 (6.9)	3 (3.4)	12 (6.8)
Ischemic heart disease, <i>n</i> (%)	61 (17)	15 (17)	33 (19) 0
Diabetes, <i>n</i> (%)	88 (24)	18 (20)	42 (24)
Renal failure, <i>n</i> (%)	36 (9.9)	6 (6.8)	18 (10)
COPD, <i>n</i> (%)	60 (17)	9 (10)	35 (20)
Malignancy, <i>n</i> (%)	68 (19)	18 (20)	28 (16)
APACHE II, mean (SD)	26 (22–32)	28 (24–33)	25 (20–32)
White blood cells, mean (SD)	14 (7.8–21.1)	15 (7.1–22.4)	13 (8–19)
Lactate, mmol/L, mean (SD)	1.7 (0.8–3.6)	2.2 (1.2–4.7)	1.5 (0.0–2.9)
Temperature, °C, mean (SD)	38 (37–38)	38 (37–38)	37 (37–38)
Heart rate bpm, mean (SD)	100 (88–115)	100 (92–114)	100 (85–117)
Systolic blood pressure, mm Hg, mean (SD)	108 (98–120)	106 (98–120)	108 (98–120)
MAP, mean (SD)	72 (67–78)	72 (68–77)	72 (67–78)
Respiratory rate, mean (SD)	19 (15–24)	19 (15–23)	19 (14–25)
Norepinephrine max dose day 1, mean (SD)	15 (9–26)	16 (9.2–29.1)	14 (8–25)
Pao2/Fio2 baseline, mean (SD)	194 (143–260)	200 (150–273)	188 (139–249)
Site of infection lung, <i>n</i> (%)	116 (44.1)	37 (42.0)	79 (50.5)
Site of infection abdominal	69 (26.2)	17 (19.3)	52 (29.5)
Other site of infection	73 (27.8)	31 (35.2)	42 (23.9)
Gram positive bacteria, <i>n</i> (%)	121 (46.4)	51 (58.0)	70 (39.8)
Gram negative bacteria, <i>n</i> (%)	79 (33.8)	33 (37.5)	46 (26.1)
Mixed, <i>n</i> (%)	29 (16.7)	4 (4.5)	25 (22.8)
Fungi, <i>n</i> (%)	24 (9.1)	4 (5.4)	20 (11.4)
28-day mortality	104 (29)	22 (25)	52 (30)
90-day mortality	136 (37)	28 (32)	69 (39)

MAP, mean arterial pressure; COPD, chronic obstructive pulmonary disease; gram-positive bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, Group B *Streptococci*, *Streptococcus species*, *Enterococcus faecalis*, *Propionibacteria*, *Clostridium*; gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, *Enterobacteriaceae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Bacteroides fragilis*.

For clustering, the *hclust* method was used for hierarchical clustering with method parameter = *complete* and *euclidean* dissimilarity matrix using transformed cytokine concentrations. Visualization was done with *heatmap* function. The *t* test for comparison of subgroups used Welch's method as above.

We also separated the 39 different cytokines into 6 groups according to their proposed mode of action as described by us previously [27, 28].

Results

Patient Demographics and Microbiology

Patients were typical of septic shock (e.g., mean age 63 years, predominantly male [60%]; Table 1). The most common site of infection was the lungs followed by abdomen in

both patients with positive blood cultures and positive cultures at other sites, and gram-positive bacteria were more commonly isolated than gram-negative bacteria (Table 1). Of the 363 patients, 264 patients (72.7%) had at least 1 organism isolated from at least 1 site. Of these, 88 patients (33.3%) had at least 1 organism isolated from blood and 176 (66.7%) had at least 1 organism isolated from other sites, such as urine, sputum, and wounds. In the remaining analyses, culture-negative patients (*n* = 99) were excluded.

There were no significant differences between patients with positive blood cultures and patients with positive cultures from other sites except for APACHE II and lactate. The most common bacteria in the positive blood culture group were *S. aureus*, *Escherichia coli*, and *Streptococcus pneumoniae*.

Fig. 1. a Forest plot showing the distribution of baseline mean normalized (SEM) cytokine levels according to organism isolated in blood or from other sites. The overall mean normalized cytokine level was set at 0.0. *N. meningitidis* isolated from other sites had the highest and *enterococci* isolated in blood had the lowest mean cytokine levels, respectively. **b** Forest plot showing the distribution of mean normalized (SEM) cytokine levels at 24 h according to organism isolated in blood or from other sites. **c** Forest plot showing the distribution of mean normalized baseline and 24 h cytokine levels according to organism isolated in blood or from other sites. Red and black squares represent baseline and 24 h samples, respectively. p values are shown for the comparison of the baseline with the 24 h mean normalized cytokine levels after FDR correction.

Table 2.**a.** Significant differences in cytokine levels between blood culture positive and any culture sites positive

All culture positives	Blood culture positives									
	<i>N. meningitidis</i> (-) blood	<i>N. meningitidis</i> (-) other site	<i>K. pneumoniae</i> (-) blood	<i>E. coli</i> (-) blood	Other gram negatives (-) blood	<i>S. pneumoniae</i> (+) blood	<i>S. aureus</i> (+) blood	<i>E. faecalis</i> (+) blood	Other gram positives (+) blood	<i>Fungus</i> blood
<i>N. meningitidis</i> (-) blood	NA	-	-	-	-	-	-	-	-	-
<i>N. meningitidis</i> (-) other site	-	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i> (-) blood	1.65E-05	-	NA	-	-	0.000255155	-	-	-	-
<i>K. pneumoniae</i> (-) other site	5.98E-33	5.83E-20	3.94E-16	3.94E-16	6.28E-23	5.41E-32	3.28E-12	1.86E-12	5.82E-18	1.99E-05
<i>E. coli</i> (-) blood	1.31E-16	0.00351	NA	NA	7.88E-05	7.14E-16	-	-	0.0961	-
<i>E. coli</i> (-) other site	9.82E-10	0.00596	-	-	0.00123	1.35E-08	-	-	-	-
Other gram negatives (-) blood	1.08E-05	-	-	-	NA	0.000214	-	-	-	-
<i>S. pneumoniae</i> (+) blood	-	-	-	-	-	NA	-	-	-	-
<i>S. pneumoniae</i> (+) other site	6.39E-06	-	-	-	0.0490	4.91E-05	-	-	-	-
<i>S. pyogenes</i> (+) blood	6.99E-10	0.0312	-	-	0.00656	1.52E-08	NA	-	-	-
<i>S. pyogenes</i> (+) other site	0.00185	-	-	-	-	0.00258	-	-	-	-
<i>S. aureus</i> (+) blood	6.56E-26	1.44E-07	0.00123	0.00123	4.35E-12	1.43E-28	-	NA	1.78E-05	-
<i>S. aureus</i> (+) other site	8.21E-42	4.49E-20	3.06E-21	3.06E-21	1.86E-30	1.35E-50	1.52E-08	3.12E-16	6.57E-19	-
<i>E. faecalis</i> (+)	1.33E-29	9.97E-20	4.78E-16	4.78E-16	1.67E-21	2.38E-28	1.26E-13	4.84E-13	7.39E-18	1.96E-07
Other gram positives (+) blood	2.42E-09	-	-	-	-	6.45E-08	-	-	NA	-
<i>Fungus</i> blood	7.93E-28	2.13E-11	1.32E-07	1.32E-07	3.54E-15	1.40E-27	0.000114	0.000496	2.27E-09	NA
<i>Fungus</i> other site	2.04E-33	4.53E-13	2.43E-10	2.43E-10	5.82E-20	3.35E-38	0.000137	3.20E-05	3.72E-11	-
Number of significant differences	14	9	6	6	10	14	5	0	7	2

b. Significant differences in cytokine levels between other sites positive and all positives

All culture positives	Other site positives									
	<i>N. meningitidis</i> (-) blood	<i>N. meningitidis</i> (-) other site	<i>K. pneumoniae</i> (-) blood	<i>E. coli</i> (-) blood	Other gram negatives (-) blood	<i>S. pneumoniae</i> (+) blood	<i>S. aureus</i> (+) blood	<i>E. faecalis</i> (+) blood	Other gram positives (+) blood	<i>Fungus</i> blood
<i>N. meningitidis</i> (-) blood	-	-	-	-	-	-	-	-	-	-
<i>N. meningitidis</i> (-) other site	NA	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i> (-) blood	8.75E-07	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i> (-) other site	1.69E-23	NA	5.98E-08	5.98E-08	7.45E-08	2.96E-23	0.000247	1.01E-07	-	-
<i>E. coli</i> (-) blood	5.42E-11	-	-	-	-	1.91E-05	-	-	-	-
<i>E. coli</i> (-) other site	1.34E-10	-	NA	NA	-	0.000434	-	-	-	-
Other gram negatives (-) blood	1.20E-06	-	-	-	-	-	-	-	-	-
<i>S. pneumoniae</i> (+) blood	0.00810	-	-	-	-	-	-	-	-	-
<i>S. pneumoniae</i> (+) other site	1.14E-07	-	-	-	NA	0.0254	-	-	-	-
<i>S. pyogenes</i> (+) blood	5.25E-10	-	-	-	-	0.00221	-	-	-	-
<i>S. pyogenes</i> (+) other site	6.87E-06	-	-	-	-	NA	-	-	-	-
<i>S. aureus</i> (+) blood	5.19E-13	-	-	-	-	6.97E-12	-	-	-	-
<i>S. aureus</i> (+) other site	2.23E-17	-	-	-	-	1.45E-26	NA	0.000426	-	-
<i>E. faecalis</i> (+)	1.48E-25	-	-	-	0.000325	4.75E-22	2.43E-06	2.62E-09	-	-
Other gram positives (+) blood	7.48E-09	-	-	-	8.24E-10	0.0261	-	-	-	-
<i>Fungus</i> blood	8.21E-17	-	-	-	-	0.00720	-	-	-	-
<i>Fungus</i> other site	1.90E-15	-	-	-	0.0143	1.33E-18	-	NA	-	-
Number of significant differences	15	0	5	5	5	11	2	3	3	2

p values of *t* tests between the pathogen in the upper heading compared to the pathogens listed on the side. *p* values have been adjusted for multiple testing using false discovery rate (FDR) correction. The bottom rows indicate the number of significantly different categories in the table. To simplify the table, values are reported for the cytokines of left-hand side pathogen level < cytokines of the top pathogen.

Fig. 2. Heat maps with cytokines classified into 6 functional groups showing blood culture at baseline. Red denotes higher and yellow denotes lower plasma mean normalized cytokine levels. Other+, indicates gram-positive organisms other than *S. pneumoniae*, *S. aureus*, *S. pyogenes*, and *E. faecalis*. Other-, indicates gram-negative organisms except *N. meningitidis*, *E. coli*, and *K. pneumoniae*. The number of isolates for each pathogen isolated from blood was *Candida* $n = 9$, *S. aureus* $n = 25$, *S. pyogenes* $n = 7$, *S. pneumoniae* $n = 9$, Other G+ $n = 3$, *E. coli* $n = 17$, *K. pneumoniae* $n = 7$, *N. meningitidis* $n = 4$, and Other G- $n = 7$.

Plasma Cytokine Levels at Baseline and 24 h According to Organism

Patients with the gram-negative *Neisseria meningitidis* ($n = 7$) and the gram-positive *S. pneumoniae* ($n = 20$) isolated from blood had the highest mean cytokine levels both at baseline and at 24 h (Fig. 1a, b). Patients with the gram-positive *Enterococcus faecalis* ($n = 7$) and the fungus *Candida* species ($n = 19$) in blood cultures had the lowest mean cytokine levels. Of the 8 different pathogens isolated from blood, patients with all but *E. faecalis* and *Candida* had higher mean cytokine values at baseline compared to at 24 h (Fig. 1c). Of the patients with gram-negative pathogens, patients who had *N. meningitidis* and *Klebsiella pneumoniae* ($n = 18$) isolated had significantly higher cytokine levels at baseline compared to at 24 h ($p_{\text{adj}} = 0.01$ and 0.02 , respectively). In contrast, patients with *E. faecalis* isolated had significantly lower cytokine levels at baseline versus 24 h ($p_{\text{adj}} < 0.01$).

Different organisms had significantly different cytokine levels (Table 2a, b); in these tables, the results of comparisons of 2 groups are shown (top category vs. side category) with number of significant differences in the bottom row. For example, patients with cultures positive for *K. pneumoniae* had significantly lower cytokine levels

than *N. meningitidis* in blood ($p_{\text{adj}} = 1.65\text{E-}5$, first column third row of Table 2a). Patients with *N. meningitidis* and *S. pneumoniae* isolated from blood had significantly higher mean normalized cytokine levels compared to all other pathogens isolated from blood or other sites except for *N. meningitidis* isolated from sputum (*N. meningitidis*, other site Table 2b).

Plasma Cytokine Clusters According to Organism

Heat maps were constructed that showed that different organisms of the same gram type elicited very different cytokine responses that did not cluster together (Fig. 2a, online suppl. Fig. 1a–c, for all online suppl. material, see www.karger.com/doi/10.1159/000500418). We found 2 larger clusters in both blood and other sites at baseline and 24 h (Fig. 2a, online suppl. Fig. 1a–c). The gram-negative *N. meningitidis* elicited the highest response and clustered with the gram-positive *S. pneumoniae* when isolated from blood cultures (Fig. 2a, online suppl. Fig. 1a). In blood and other culture sites, *E. faecalis* and *Candida* consistently had the lowest cytokine responses.

The 39 cytokines were separated into 6 groups according to their proposed mode of action [27, 28]. Pro- and anti-inflammatory cytokines clustered together at base-

line and at 24 h (Fig. 2a, online suppl. Fig. 1a–c) with the exception that *Streptococcus pyogenes* ($n = 6$) switched to the high cytokine cluster trading place with *K. pneumoniae* (Fig. 2a, online suppl. Fig. 1a–c).

In blood cultures, *N. meningitidis* and *S. pneumoniae* plasma cytokine levels were significantly higher than all other species, while *E. faecalis* had significantly lower plasma cytokine levels compared to all other organisms in blood or other sites overall (Table 2a).

Discussion

We found that the inflammatory response at baseline and at 24 h differed according to organism, as opposed to gram type, in patients with septic shock, both among organisms isolated from blood or from other sites in patients who had septic shock. *N. meningitidis*, *S. pneumoniae*, and *K. pneumoniae* elicited the highest inflammatory response as indicated by significantly higher plasma cytokine levels; the gram-positive *E. faecalis*, the gram-negative *E. coli*, and the fungus *Candida* were associated with the lowest plasma cytokine levels. Furthermore, the inflammatory response at baseline in patients with positive blood cultures clustered into 2 distinct groups of high and low plasma cytokine levels. For most organisms, the baseline plasma cytokine levels were higher than at 24 h except for *E. faecalis* in blood and *S. aureus* and *K. pneumoniae* from other sites.

Surprisingly, the cytokine response was very similar in gram-positive and gram-negative organisms. All organism types are ligands for various Toll-like receptors that signal comparable inflammatory responses. Gram-positive organisms activate the immune system through TLR-2, whereas gram-negative organisms stimulate TLR-4 [29]. Our findings suggest that there is another more level of organism-specific control of the inflammatory response in septic shock as assessed by plasma cytokines because we found significant differences in the inflammatory response according to specific organism.

The cytokine responses differed between patients with positive blood versus other culture sites. One possible explanation is that blood culture positivity elicits a different immune response because of the need for a more rapid and potent sustained response than when organisms are not present in blood. Another possible explanation is that the organisms isolated from other nonblood sites were not infecting pathogen but were colonizing organisms.

Plasma cytokine levels were higher at baseline and decreased by 24 h for most organisms (except for *E. faecalis*

in blood and *S. aureus* and *K. pneumoniae* from other sites), aligning with our prior finding that survivors have greater decreases of cytokine levels than nonsurvivors [27, 28].

Our findings that gram type is not associated with the level of inflammatory response are consistent with studies showing that there were no differences between patients with gram-positive versus gram-negative infections and the presence of sepsis-associated coagulopathy [16].

Strengths of our study include the multicenter design, a large sample size, detailed microbiology results from clinical laboratories (a feature of pragmatic design), measurement of 39 plasma cytokines, chemokines, and growth factors, and use of hierarchical and functional cytokine class clustering.

There are limitations of our study. First, we did not measure organism load and that could have differed according to specific organism, thereby altering the rate and degree of cytokine response. Second, we used clinically available cultures from the clinical sites in VASST and that adds some variability in terms of both when cultures were taken (this was not protocolized) and laboratory procedures for organism detection. The study is retrospective, and we did not have access to the information on the clinical judgment whether a pathogen was considered relevant or not and we did not have information regarding initial adequate antimicrobial treatment and source control. Another limitation is that sicker patients have higher cytokine levels independent of organism or that patients who have community-acquired sepsis (e.g., *N. meningitidis*) have higher cytokine levels and are seen earlier in their illness. In the VASST study, there was community- and hospital-acquired sepsis, but the current study was not adequately powered to analyze these subgroups further. We have not analyzed or evaluated the issue of mixed isolates herein because we were interested in the individual organism associations with outcomes. Finally, we do not have specific information on the laboratory methods for microbial diagnostics which were the standard accredited clinical hospital laboratory methods at each site.

Also, we measured plasma cytokines at baseline and at 24 h, so our interpretations are limited to early responses in plasma according to organism in septic shock. We measured 39 cytokines in plasma at baseline and at 24 h so cannot imply associations of organism and other inflammatory markers at later time points. Despite our large sample size, the subgroups of organism type were too small to assess the relationship of specific organism and mortality.

In conclusion, the inflammatory response at baseline and at 24 h in human septic shock differed according to organism, as opposed to gram type. Patients with septic shock display unique early cytokine profiles according to organism type but not gram type. The gram-negative *N. meningitidis*, the gram-positive *S. pneumonia*, and the gram-negative *K. pneumoniae* elicited the highest cytokine responses. In contrast, the gram-positive *E. faecalis*, the gram-negative *E. coli*, and the fungus *Candida* were associated with the lowest plasma cytokine levels. Larger trials that modulate the inflammatory response in septic shock may benefit by evaluating the therapies in subgroups according to the specific organism rather than according to organism gram type.

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Appendix

In addition to the authors, the following persons and institutions participated in the VASST trial: *Writing Committee*: J.A. Russell (chair), K.R. Walley, A.C. Gordon, C. Holmes Boulton, J.T. Granton, P.C. Hebert, D.J. Cooper, S. Mehta, J. Singer, D.J. Cook, J.J. Presneill, M.M. Storms; *Executive Committee*: J.A. Russell (chair), K.R. Walley, C.L. Holmes Boulton, J.T. Granton, P.C. Hebert, D.J. Cooper, S. Mehta, J. Singer, A.C. Gordon, M.M. Storms (project coordinator), S. Jones (administrative assistant); *Management Committee*: J.A. Russell (chair), M.M. Storms (project coordinator), K.R. Walley, C.L. Holmes Boulton, J. Singer, A.C. Gordon, S. Jones (administrative assistant); *DSMB*: G.R. Bernard (chair), A.S. Slutsky, G.A. Wells; *CIHR*: A. Gasparini; *Data Management*: J. Singer, B. Savage, D. Ayers, R. Woods, K. Wu, M. Maralit; *Monitoring*: L. Smith, K. Foley, A. Suri, M. Steinberg, B. Howe, P. Galt, A. Higgins, M.M. Storms; *Laboratory*: M.E. LeBlanc, A.M. Sutherland, A. Sham, A. McLeod.

Clinical Centers and VASST Investigators: Canada, British Columbia; *St. Paul's Hospital* – J.A. Russell, K.R. Walley, D.R. Dorscheid, M. Hameed, L. Lazosky, S. Helderweirt, K. Foley, C. Honeyman, T. Terins; *Vancouver General Hospital* – D. Chittock, J. Ronco, L. Smith, S. Logie; *Royal Jubilee Hospital* – G. Wood, F. Auld; *Kelowna General Hospital* – C. Holmes Boulton, V. Stedham, M. Mantle, L. Fox, G.D. McCauley, J.D. Rolf, C. Erbacher; *Richmond General Hospital* – G. Martinka, S. Goulding, S. Silverwood, L. Leung; *Royal Columbian Hospital* – S. Keenan, J. Murray, M. Van Osch, Manitoaba; *St. Boniface Hospital* – B. Light, M. Dominique; *Winnipeg Health Science Centre* – P. Gray, R. Stimpson, S. Rosser, D. Bell, W. Janz. Ontario; *Ottawa Hospital, General Campus* – P.C. Hebert, T. McArdle, I. Watpool; *UHN* – Toronto Gen-

eral & Toronto Western Hospitals – J.T. Granton, M. Steinberg, A. Matte-Martyn; *St. Joseph's Hospital* – D.J. Cook, E. McDonald, F. Clarke, A. Tkaczyk, N. Zytaruk; *Mount Sinai Hospital* – S. Mehta, T. Stewart, A. Suri, C. Martinez-Motta, R. MacDonald, V. Sivanantham; *Ottawa Hospital, Civic Campus* – R. Hodder, J. Foxall, M. Lewis; *St. Michael's Hospital* – M. Ward, C. Dos Santos, J. Friedrich, D. Scales, O. Smith, I. DeCampos, A. Richards, H. Michalopoulos, U. Bakshi; *Sunnybrook and Women's College Health Science Centre* – W. Sibbald (deceased), T. Smith, K. Code, B. Bojilov, C. Dale, M. Keogh; *Hamilton Health Sciences Centre* – M. Meade, L. Hand; *London Health Sciences Centre* – C. Martin, J. Kehoe, V. Binns; *Sudbury Regional Hospital* – M. Mehta, M. McGuire; *Charles LeMoine Hospital* – G. Poirier, L. Provost; *Windsor – Hotel Dieu Hospital* – J. Muscedere, C. Diemer. Australia: Victoria; *Alfred Hospital* – D.J. Cooper, V. Pellegrino, A. Hilton, S. Morrison, C. Weatherburn, K. Moulden, C. Harry; *Royal Melbourne* – J.J. Presneill, M.S. Robertson, J.F. Cade, B.D. Howe, D.K. Barge, C.A. Boyce, F. Healy; *Monash Medical Center* – C. Wright, D. Weyandt, J. Barrett, C. Walker, P. Galt, S. Burton. Western Australia; *Royal Perth Hospital* – G. Dobb, S. Perryman, J. Chamberlain, L. Thomas. South Australia; *Flinders Medical Centre*, A. Bersten, L. Daly, T. Hunt, D. Wood. USA: *Mayo Hospital* – B. Patel, J. Larson, M. Rady, G. LeBrun, E. Boyd, R. Rush.

Disclosure Statement

Dr. James A. Russell reports patents owned by the University of British Columbia (UBC) that are related to PCSK9 inhibitor(s) and sepsis and related to the use of vasopressin in septic shock. Dr. James A. Russell is an inventor on these patents. Dr. James A. Russell is a founder, director, and shareholder in Cyon Therapeutics Inc. (developing a sepsis therapy (PCSK9 inhibitor)). Dr. James A. Russell has share options in Leading Biosciences Inc. Dr. James A. Russell is a shareholder in Molecular You Corp. Dr. James A. Russell reports receiving consulting fees in the last 3 years from: Asahi Kasei Pharmaceuticals of America (AKPA; developing recombinant thrombomodulin in sepsis). La Jolla Pharmaceuticals (developing angiotensin II; Dr. Russell chaired the DSMB of a trial of angiotensin II from 2015 to 2017) – no longer actively consulting. Ferring Pharmaceuticals (manufactures vasopressin and was developing selepressin) – no longer actively consulting. Cubist Pharmaceuticals (now owned by Merck; formerly was Trius Pharmaceuticals; developing antibiotics) – no longer actively consulting. Leading Biosciences (was developing a sepsis therapeutic that is no longer in development) – no longer actively consulting. Grifols (sells albumin) – no longer actively consulting. CytoVale Inc. (developing a sepsis diagnostic) – no longer actively consulting. Dr. James A. Russell reports having received an investigator-initiated grant from Grifols (entitled “Is HBP a mechanism of albumin's efficacy in human septic shock?”) that is provided to and administered by UBC.

The other authors have no conflicts of interest.

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