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Inflammasome Expression and Cytomegalovirus Viremia in Critically Ill Patients with Sepsis

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Abstract

Background—CMV viremia is a contributor to poor outcomes in critically ill patients with sepsis.

Objectives—To assess the expression levels of genes encoding inflammasome-related proteins in the development of CMV viremia in critically ill patients with sepsis.

Study Design—A cohort of CMV-seropositive critically ill patients with sepsis due to bloodstream infection underwent weekly testing for CMV viremia. Blood samples to evaluate *mRNA* levels of genes encoding *CASP1*, *ASC*, *NLRP1*, *NLRP3*, and *NLRP12* were collected at the time of enrollment. Clinical outcomes were assessed at 30 days or until death/discharge from ICU.

Results—CMV viremia was documented in 27.5% (8/29) of the patients, a median of 7 days after the onset of bacteremia. Patients with sepsis who developed CMV viremia had higher *CASP1* although this was not statistically significant (relative mean 3.6 vs 1.8, $p=0.13$). Development of high grade CMV viremia however, was significantly associated with *CASP1*; septic patients who

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developed high grade CMV viremia had significantly higher *CASP1* than all other patients (relative mean 5.5 vs 1.8, $p=0.016$).

Conclusions—These data document a possible involvement of inflammasomes in the pathogenesis of CMV. Regulating the host immune response by agents that target these genes may have implications for improving CMV-related outcomes in these patients.

Keywords

Cytomegalovirus; sepsis; inflammasome; Caspase-1; ASC; NLRP1; NLRP3; NLRP12

BACKGROUND

Cytomegalovirus (CMV) is a member of the herpesvirus family and a significant human pathogen. CMV seropositivity indicative of latent infection, is common in general population with rates of ~60–70% in the United States and higher in other parts of the world (Staras, et al. 2006). The virus establishes latency with lifelong persistence in infected individuals without overt or adverse sequelae. Latent infection, however, has the potential to reactivate and produce lytic virus. Although frequently documented in immunosuppressed patients, CMV viremia is exceedingly rare in immunologically competent individuals (Ho 2008). However, it has been recognized that critically ill patients who are otherwise immunocompetent are also at risk for CMV and that reactivation of CMV in these patients is a contributor to poor outcomes (Frantzeskaki, et al. 2015; Limaye, et al. 2008; von Müller, et al. 2006). CMV viremia occurs in 15–30% of these patients, and sepsis-associated changes in the host innate immunity and upregulation of inflammatory cytokines are proposed to be the basis for the development of viremia (Limaye, et al. 2008; von Müller, et al. 2006).

NOD-like receptors (NLR) are intracellular sensors to detect microbes and danger molecules, and enhance innate immune responses (Ting and Davis 2005; Ting, et al. 2006). Some NLR proteins are involved in the assembly of a cytosolic multiprotein complex termed the “inflammasome.” Components of inflammasomes typically include a NLR (e.g., NLRP1 or NLRP3, serving as a sensor), pro-caspase-1 (CASP1), and apoptosis-associated speck-like protein (ASC), an adapter protein bridging between NLR and pro-caspase-1 (Fahy, et al. 2008; Mariathasan and Monack 2007; Master, et al. 2008; Ryan and Kastner 2008; Sutterwala, et al. 2007). Once the inflammasome complex is formed, pro-caspase-1 goes through self-cleavage to become active. A key function of caspase-1 is to cleave pro-IL-1 β and pro-IL-18 to mature inflammatory cytokines IL-1 β and IL-18, which are released to extracellular spaces by pyroptosis, an inflammatory cell death (Lamkanfi, et al. 2007; Mariathasan and Monack 2007). IL-1 β is one of the most potent proinflammatory cytokines for the generation of systemic and localized responses to sepsis (Arend, et al. 2008; Mariathasan and Monack 2007; Sidiropoulos, et al. 2008). Thus, inflammasomes form molecular scaffolding and function as critical inducers of inflammatory responses (Fahy, et al. 2008; Sutterwala, et al. 2007).

Although inflammation is an essential component of protective responses to pathogens, proinflammatory pathology may have detrimental effects including reactivation of CMV (Cook, et al. 2006; Hummel and Abecassis 2002). Detection of IL-1 β through IL-1 receptor,

which activates NF- κ B, leads to CMV reactivation (Löser, et al. 1998; Sambucetti, et al. 1989). However, the contribution of inflammasomes in the pathogenesis of CMV is not fully understood. The goals of the study herein were to assess expression levels of genes encoding inflammasome-related proteins -i) in the overall population of critically ill patients with sepsis, ii) as predictors of outcomes in septic patients, iii) and development of CMV viremia in a prospectively followed cohort of critically ill patients with sepsis.

STUDY DESIGN

Study population

We performed a prospective and observational study involving critically ill patients with sepsis due to bloodstream infections. The clinical characteristics and patient outcomes associated with CMV viremia in these patients have recently been reported (Osawa, et al. 2016). Bloodstream infections in the patients were conducted between December 2008 and June 2014 at the intensive care units of two University-affiliated medical centers (Osawa, et al. 2016). This study was approved by the institutional review board, and informed consent in a written form was obtained from the patients or their legally authorized representatives. Inclusion criteria included i) age $\geq$ 18 years and ii) hospitalization in the ICU at the time of or within 48 hours of the onset of bloodstream infection. Patients with sepsis met previously defined International Sepsis Definition criteria (Levy, et al. 2003) and had microorganism-positive blood culture with at least a single microbial species other than coagulase-negative staphylococci, the *Bacillus* species and diphtheroids based on the criteria for bacteremia by Centers for Disease Control and Prevention (Garner, et al. 1988). Bacteremia due to intravascular catheters or bacteremia without a documented source was regarded as primary bacteremia (Garner, et al. 1988). We considered patients to be immunocompetent if they had no known or overt evidence of immunosuppression. Patients were excluded if they were immunosuppressed as a result of; organ transplantation or hematopoietic stem cell transplantation, human immunodeficiency virus infection, receipt of antiviral medications with activity against CMV (i.e. ganciclovir or valganciclovir), and receipt of iatrogenic immunosuppressive agents, such as prednisone at a dose of > 0.5 mg/kg/day for at least 2 weeks prior to onset of sepsis, tumor necrosis factor antagonists, methotrexate, cancer chemotherapy within 4 weeks prior to the onset of sepsis.

Clinical assessments

Characteristics of the study population included demographic data, medical history, clinical and laboratory data were abstracted systematically in a data collection form. Organ failure was assessed upon enrollment into the study (considered as baseline) using Sequential Organ Failure Assessment (SOFA) score on a scale ranging from 0 to 4 for each of the six major organ systems, for an aggregate score of 0 to 24, with higher scores indicating more severe organ dysfunction (Vincent, et al. 1996). Multiple organ failures were defined as ≥ 2 organ failure. Patients were followed for 30 days from the date of positive blood culture or until death or discharge from the ICU.

Laboratory assessments

CMV assays—After obtaining informed consent, a screening test for CMV antibody to determine CMV serostatus was performed using a commercially available immunoassay per manufacturer's instructions (Bio Merieux Vidas CMV IgG). Patients seropositive for CMV were enrolled and underwent weekly assessment for CMV viremia for 30 days from the date of positive blood culture or until death or discharge from the ICU. CMV assays were performed using a non-commercial whole blood quantitative real-time PCR as previously reported (Sanghavi, et al. 2008; Sun, et al. 2010). Briefly, DNA was extracted from 200µl of whole blood into 150µl elution volume, of which 5µl was used per PCR reaction. The primer sequences tested were: US17 forward 5'-CGATCAAGAACGCGATAACG-3'; US17 reverse 5'-ACCGTCGATGGCAGGTCAT-3'; US17 probe 6FAM-CGA TCA CAA ACA GCG-MGB; UL54 forward 5'-CGCAGTCTACCTCGATATCACAA-3; UL54 reverse 5'-TGCTCCGTGAATCGTTACGA-3'; UL54 probe 6FAM-CCCTGCTGCCGCCA-MGB. Each real-time PCR was performed in 25µl reaction volume and consisted of 1×Taqman PCR master mix (Applied Biosystems, CA), 0.5µM of forward and reverse primers, 0.2 µM of probe and 5µl of extracted DNA. Viral load represented CMV DNA copies/mL. The assay's range for the detection of CMV was 50-10⁶ copies/mL. High-grade viremia was defined as CMV DNA PCR copies > 500 copies/mL (Sanghavi, et al. 2008). The results of CMV PCR assays were not used for clinical care.

Sample collection and processing—Blood samples from patients with sepsis were collected for the assessment of *CASP1*, *ASC*, *NLRP1*, *NLRP3*, and *NLRP12* mRNA levels were collected immediately upon enrollment into the study. Approximately 10 mL of whole blood samples were collected in EDTA-containing tubes and filtered immediately through LeukoLOCK Total RNA Isolation System (Thermo Fisher Scientific, Waltham, MA) that captures the total leukocyte population while eliminating plasma, platelets and red blood cells. The filter was flushed with a phosphate-buffered saline solution to remove residual red cells and then with *RNAlater*^(R) to stabilize leukocyte RNA. The filter with stabilized leukocytes was sealed and stored at -80°C per manufacturer's instructions.

Evaluating mRNA levels by qPCR—Quantitative PCR was performed as previous performed (Inoue, et al. 2016; Kanayama, et al. 2015). cDNA was prepared from mRNA by reverse transcription (QuantiTect Reverse Transcription Kit, Qiagen). mRNA expression levels were determined by real-time PCR using the *Ct* method with SYBRTM Green master mixes (Applied Biosystems) with *ACTB* an internal control. Samples were run triplicate. Primer sequences used in analyses were *CASP1* (forward, 5'-GGATATGGAAACAAAAGTCGGC-3'; reverse 5'-CATTGTCATGCCTGTGATGTC-3'), *ASC* (forward, 5'-TCACCGCTAACGTGCTG-3'; reverse 5'-TGGTCTATAAAGTGCAGGCC-3'), *NLRP1* (forward, 5'-CCGCTGACCCCACTTTATATG-3'; reverse 5'-CAACGTAGAACTCCGAGAACAG -3'), *NLRP3* (forward, 5'-GTGTTTCGAATCCCACTGTG-3'; reverse 5'-TCTGCTTCTCACGTACTTTCT-3'), *NLRP12* (forward, 5'-TGACAGGAAATGCACTGGAG-3'; reverse 5'-GGTTCACACTGAGAGTTGAGG-3'), and *ACTB* (forward, 5'-ACCTTCTACAATGAGCTGCG-3'; reverse 5'-CCTGGATAGCAACGTACATGG-3'). PCR reaction was initiated with a step at 95 C for

10 min and performed with 40 cycles of denaturation at 95 C for 15 sec and annealing/extension at 60 C for 1 min. A majority of the qPCR results showed the range in the Ct value between 17 and 23. The highest Ct values detected in this entire study was 26.54. By confirming the kinetics of relative fluorescent levels vs. PCR cycles, we concluded that all the samples used in this study had mRNA levels above the detection limit. We also evaluated the RQ-Min and RQ-Max values from technical triplicate [$RQ-Min = 2^{-(Ct + T * SD(Ct))}$, $RQ-Max = 2^{-(Ct - T * SD(Ct))}$; RQ-MIN and RQ-MAX constitute the acceptable error for a 95% confidence limit according to Student's t test]. When either an RQ-MIN or RQ-MAX level was 25% less or more, respectively, of the corresponding 2^{-Ct} value, we analyzed the sample until the technical variation achieved satisfactory within the range. mRNA levels are shown with relative values based on those from a randomly selected patient sample in this cohort.

Statistical analyses

Stata/SE (College Station, TX, version 14.2) was used for statistical analysis shown in all the tables (values for mean and P-values). See the subsection "Evaluating mRNA levels by qPCR" for the method of analyzing qPCR data alone.

RESULTS

The patient population comprised 29 critically ill patients with sepsis. Table 1 depicts the demographic and clinical data, underlying conditions, and sources of bacteremia in these patients.

Expression of genes encoding inflammasome components in all patients

mRNA levels of genes encoding inflammasome components based on patient's clinical characteristics, comorbid conditions and severity of illness (APACHE score) are shown in Table 2. Critically ill patients who developed sepsis following recent surgery had significantly lower *ASC* mRNA levels than those without surgery (relative mean 1.60 vs. 2.64, $p=0.025$). The severity of illness as assessed by APACHE scores also correlated significantly with *NLRP3* mRNA levels; patient's with high APACHE score had significantly higher *NLRP3* mRNA levels than those with lower APACHE scores (relative mean 7.73 vs 3.15, $p=0.049$). *NLRP1* mRNA levels were lower in patients with malignancy (relative mean 6.02 vs 1.29, $p=0.080$). Patients with primary bacteremia as a source of sepsis had higher *NLRP1* (relative mean 1.38 vs 0.72, $p=0.070$) and lower *ASC* mRNA levels (1.82 vs 2.61, $p=0.080$) than those with bacteremia due to other sources although the difference was not statistically significant. Diabetic patients had lower *NLRP3* (relative mean 3.1 vs 7.46, $p=0.060$) and *NLRP12* (relative mean 0.56 vs 2.45, $p=0.059$) mRNA levels than non-diabetic patients. No association was documented between *CASP1*, *ASC*, *NLRP1*, *NLRP3* or *NLRP12* with age, renal dysfunction, or other comorbidities (Table 2).

Expression of genes encoding inflammasome components and CMV viremia

Reactivation of CMV or viremia was documented in 27.5% (8/29) of the patients with sepsis. CMV viremia developed a median of 7 days, range 2–15 days after the onset of bacteremia. Only *CASP1* mRNA levels showed an association with CMV viremia (Table 3).

Patients with sepsis who developed CMV viremia had higher *CASP1* mRNA levels although this was not statistically significant (relative mean 3.6 vs. 1.8, $p=0.13$). Development of high-grade CMV viremia, however, was significantly associated with *CASP1* mRNA levels; septic patients who developed high-grade CMV viremia had significantly higher *CASP1* mRNA levels than those without high-grade CMV viremia (relative mean 5.5 vs. 1.8, $p=0.016$). No correlation was found between the development of CMV viremia and expression of other genes tested in this study (Table 3).

Expression of genes encoding inflammasome components and patient outcomes

Table 4 depicts the key outcomes in the study population, *i.e.*, mortality and/or multi-organ failure. Expression levels of *CASP1*, *ASC*, *NLRP1*, *NLRP3* or *NLRP12* did not correlate with mortality (Table 4). When the primary end-point of the parent study (mortality and/or MOF) was considered, patients with death/MOF compared to those alive without MOF had higher mRNA levels of *CASP1* (relative mean 2.9 vs 1.70, $p=0.158$) and *NLRP12* (relative mean 2.1 vs 0.65, $p=0.145$), however the difference was not statistically significant. Results were similar when MOF alone was considered; mRNA levels of *CASP1* (relative mean 2.9 vs 1.70, $p=0.158$) and *NLRP12* (relative mean 2.1 vs. 0.65, $p=0.145$) did not differ between patients with or without MOF.

DISCUSSION

Sepsis is a contributor to poor outcomes in patients, increased hospital costs, and resource utilization. An estimated 751,000 cases of sepsis occur per year in the United States with overall mortality rate ranging from 20–50% in those who require ICU care (Angus, et al. 2001). Limited data exist on gene expression of inflammasome component proteins in patients with sepsis (Fahy, et al. 2008; Giamarellos-Bourboulis, et al. 2011) and to the best of our knowledge, no study has reported gene expression levels of inflammasome components in septic patients with CMV. Our data show that patients who developed sepsis following a recent surgical procedure had significantly lower levels of *ASC* mRNA. The adapter protein ASC contains a caspase recruitment domain and is pivotal for inflammasome assembly in response to diverse danger signals, including pathogen-derived factors (Ellebedy, et al. 2011). Surgical stress is associated with a pro-inflammatory state due to activation of neutrophils and macrophages that are primed by circulating cytokines and chemoattractants in the injured tissue. After major trauma, maximum priming of neutrophils occurs within 3–24 hours of following which functional exhaustion and unresponsiveness of neutrophils may ensue (Botha, et al. 1995). Other studies have shown progressive downregulation instead of a biphasic response (Brochner and Toft 2009). For example, the ability of the monocytes to generate cytokines was weakened following major trauma with reduced expression of MHCII and decreased cytokine production (Brochner and Toft 2009). Additionally, the NK cell activity remained suppressed for 2–4 weeks after major trauma (Jira, et al. 1988). These responses are considered as damage control measures aimed at normalizing the innate host responses after tissue injury (Stahel, et al. 2005) and support our findings showing reduced *ASC* gene expression in patients who developed sepsis following a major surgery.

Data in animal models have shown that deficiency of caspase-1, ASC or NLRP3 facilitates the risk and severity of malignancy (Hu, et al. 2010). The role of caspase-1 in carcinogenesis is mediated through its direct effects on colonic epithelial cell proliferation and apoptosis, rather than through regulation of colonic inflammation (Hu, et al. 2011; Hu, et al. 2010). Caspase-1 is a key regulator of epithelial cell apoptosis and failure to activate it disrupted the apoptotic signaling pathways in tumor tissue and promoted tumor formation (Jarry, et al. 1999). Surgically resected colonic specimens showed that while caspase-1 was strongly expressed in normal colonic epithelium, its expression was significantly downregulated in human colonic cancer (Jarry, et al. 1999). NLRP3 likewise suppressed colorectal cancer growth by promoting NK cell mediated anti-tumor activity (Dupaul-Chicoine, et al. 2015). NLRP3 regulates carcinogenesis through the hematopoietic myeloid cells. This finding is consistent with the known biology of NLRP3 since the NLRP3 function is largely attributable to its presence in myeloid-derived cells, specifically monocytes/macrophages. Once the tumor becomes established, these create a local milieu conducive for tumor growth, angiogenesis, and metastasis (Allen, et al. 2010). Along these lines, our data show reduced *NLRP1* gene expression in septic patients with malignancy although a small number of these patients may have precluded achieving statistical significance.

A key finding of our study is the association of high-grade CMV viremia with *CASP1* mRNA levels; septic patients who developed high-grade CMV viremia had significantly higher mRNA levels than those without high-grade CMV viremia. CMV infects monocytes/macrophages, endothelial cells, epithelial cells, smooth muscle cells, fibroblasts, stromal cells, and neutrophils (Compton, et al. 2003). The virus has the ability to elicit a potent inflammatory response that is mediated via Toll-like receptor 2 (TLR2)-dependent activation of NF- κ B (Roy and Arav-Boger 2014). This transcription factor family is a central hub of signaling events and is activated by viruses, including CMV that harbors binding sites for NF- κ B (Roy and Arav-Boger 2014). NF- κ B has a key role in maintaining the balance between latency and reactivation. Thus, while CMV *per se* facilitates the production of inflammatory cytokines, activation of the *CASP1* gene expression preceded reactivation of CMV, suggesting that CMV viremia was a downstream event and sequelae of high *CASP1* mRNA levels and not vice versa.

Our data also documented an association of *NLRP3* mRNA levels with severity of illness as assessed by APACHE II score; *NLRP3* mRNA levels were significantly higher in patients with greater severity of illness. *NLRP3* gene expression is activated in response to the widest array of exogenous and endogenous stimuli, including microbial agents and other endogenous danger signals, and participate in forming the NLRP3 inflammasome complex, which processes IL-1 β and IL-18 secretion to elicit an intense inflammatory response (Guo, et al. 2015). In a previous study, *NLRP3* mRNA levels correlated significantly with Gensini score, that evaluates the severity of coronary artery disease (Wang, et al. 2014). Indeed, *NLRP3* mRNA levels correlated with the severity of not only acute illness but also with underlying illness (Wang, et al. 2014). *NLRP3* mRNA levels level remained elevated during the acute phase of the illness i.e., at 48–72 h, a time point consistent with the assessment of *NLRP3* mRNA levels in our study, with subsequent decline (Wang, et al. 2014).

This study has weaknesses that must be acknowledged. Foremost amongst these is that our sample size was small and therefore, our findings must be considered as hypotheses generating rather than proof of concept. Gene expression of inflammasome components was a sole readout of this study; thus, further studies will be required to evaluate protein expression and function of inflammasomes, *e.g.*, the activation status of inflammasomes. Also, given that our study focused on patients with sepsis due to bloodstream infections, comparison of this cohort with patients who had sepsis due to other etiologies is not available. Nevertheless, strengths of our study include prospective sample analyses, which were performed in a blinded fashion by personnel who were independent of the clinical team. More importantly, patients' samples used in this study are rare, and obtaining and analyzing such samples are challenging. Therefore, we believe that this study, although data relating to inflammasomes is solely on gene expression levels, sets a new stage for eventually elucidating functions of inflammasomes in critically ill patients from sepsis.

Despite the availability and wide use of antiviral therapy for over two decades, CMV continues to be associated with significant morbidity and poor outcomes. Additionally, anti-CMV agents are associated with significant and often therapy-limiting adverse effects. Understanding the unique role of inflammasomes in enhancing the host immune response has implications for harnessing the native immune responses to CMV for improving outcomes. Agents that target inflammasomes and surrounding signaling pathways have been successfully employed in the management of various inflammatory disorders (Osawa, et al. 2011; So, et al. 2007). Limited data also exist for the use of these agents for infectious diseases (Braun, et al. 1999). By yielding insights into the basis for approaches that modulate the expression and function of inflammasomes, data such as ours has wider relevance for optimizing the management of sepsis in critically ill patients.

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Table 1

Characteristics of the study population (n=29)

Variable	Value
Demographic data	
Age, median (IQR) ¹	63 (59–71)
Gender:	
Male	26 (89.7%)
Female	3 (10.3%)
Race:	
White	23 (79.3%)
Black	5 (17.2%)
Hispanic	1 (3.5%)
Medical history	
Recent surgery ²	10 (34.5%)
Diabetes mellitus	15 (51.7%)
Insulin dependent diabetes	8 (27.6%)
Chronic lung disease	9 (31.0%)
Renal failure	9 (31.0%)
Requirement of dialysis	7 (24.1%)
Malignancy	5 (17.2%)
Congestive heart failure	3 (10.3%)
APACHE II, median(IQR)	24 (17–29)
APACHE II >25	13 (44.8%)
Bloodstream infection source	
Primary bacteremia	12 (41.4%)
Pneumonia	2 (6.9%)
Urinary tract infection	4 (13.8%)
Cardiovascular	4 (13.8%)
Abdominal	2 (6.9%)
Skin/soft tissue	1 (3.5%)
Bone/joint	1 (3.5%)
Surgical site	3 (10.3%)
Bloodstream isolate	
<i>S. aureus</i>	5 (17.3%)
Enteric Gram-negative rod	19 (65.5%)

Variable	Value
<i>Pseudomonas</i>	1 (3.5%)
Enterococci	4 (13.8%)
Outcomes	
Died	4 (13.8%)
MOF ³	13 (44.8%)
MOF or Death	13 (44.8%)

¹IQR= Interquartile range;

²Types of surgery includes coronary artery bypass graft (3), laminectomy and fusion, abdominal aortic aneurysm repair, lobotomy and cranioplasty, vertebral tumor resection, splenectomy and pancreatectomy, fistula resection and debridement in one patient each.

³MOF= Multi organ failure

Table 2
Inflammasome gene expression in study patients stratified by underlying conditions and clinical characteristics

Variable	CASPI		ASC		NLRP1		NLRP3		NLRP12	
	Mean [SD]	p-value	Mean [SD]	p-value	Mean [SD]	p-value	Mean [SD]	p-value	Mean [SD]	p-value
Age 65	1.64 [0.74]	0.155	2.18 [1.31]	0.616	0.98 [0.94]	0.920	3.96 [4.49]	0.210	1.56 [2.77]	0.842
Age>65	3.23 [4.42]		2.42 [1.05]		1.01 [1.05]		6.96 [8.07]		1.35 [2.77]	
Recent surgery										
Yes	1.11 [0.46]	0.117	1.60 [0.99]	0.025	0.62 [0.70]	0.131	3.90 [5.12]	0.425	1.54 [3.53]	0.923
No	2.92 [3.48]		2.64 [1.16]		1.19 [1.04]		5.90 [6.83]		1.44 [2.30]	
Diabetes mellitus										
Yes	1.77 [0.85]	0.326	2.33 [1.23]	0.814	0.84 [0.86]	0.402	3.10 [3.82]	0.060	0.56 [0.49]	0.059
No	2.86 [4.14]		2.22 [1.20]		1.15 [1.08]		7.46 [7.64]		2.45 [3.70]	
Chronic lung disease										
Yes	1.83 [1.27]	0.573	1.87 [0.71]	0.226	1.36 [1.35]	0.175	5.50 [8.82]	0.869	1.83 [3.18]	0.650
No	2.51 [3.45]		2.46 [1.34]		0.82 [0.72]		5.07 [5.01]		1.32 [2.57]	
Renal failure										
Yes	1.94 [1.00]	0.671	2.82 [1.34]	0.102	0.97 [0.89]	0.957	6.00 [6.13]	0.656	0.85 [0.75]	0.417
No	2.46 [3.49]		2.04 [1.08]		0.99 [1.03]		4.85 [6.46]		1.76 [3.23]	
Dialysis										
Yes	1.86 [1.03]	0.657	2.46 [0.69]	0.652	0.89 [0.93]	0.766	5.14 [6.77]	0.975	0.60 [0.51]	0.342
No	2.44 [3.33]		2.22 [1.33]		1.02 [1.00]		5.22 [6.27]		1.75 [3.08]	
Malignancy										
Yes	1.39 [0.53]	0.457	1.92 [1.15]	0.466	0.30 [0.33]	0.080	1.29 [1.24]	0.127	0.40 [0.26]	0.343
No	2.49 [3.20]		2.36 [1.22]		1.13 [1.00]		6.02 [6.60]		1.70 [2.95]	
APACHE II 25	1.58 [0.68]	0.146	2.21 [1.33]	0.742	1.16 [1.08]	0.402	3.15 [3.40]	0.049	1.52 [2.79]	0.925
APACHE II >25	3.18 [4.25]		2.36 [1.07]		0.85 [0.87]		7.73 [8.05]		1.42 [2.74]	
Type of bacteremia:										

Variable	CASP1		ASC		NLRP1		NLRP3		NLRP12	
	Mean [SD]	p-value	Mean [SD]	p-value	Mean [SD]	p-value	Mean [SD]	p-value	Mean [SD]	p-value
Primary bacteremia	1.83 [1.15]	0.487	1.82 [1.05]	0.080	1.38 [1.27]	0.070	6.95 [8.49]	0.216	1.65 [2.73]	0.779
Yes	2.62 [3.74]		2.61 [1.22]		0.72 [0.58]		3.98 [3.93]		1.35 [2.79]	
No										

¹ Mean relative level (SD=standard deviation), p-value

² IQR=Interquartile range

Table 3

Inflammasome levels in patients with and without CMV viremia

CMV viremia versus no viremia			
Inflammasome	CMV viremia		
	Patients with CMV viremia [n=8] Mean relative level [SD]	Patients without CMV viremia [n=21] Mean relative level [SD]	P-value
<i>CASP1</i>	3.62[5.39]	1.79 [0.99]	0.138
<i>ASC</i>	2.37[1.26]	2.25[1.21]	0.816
<i>NLRP1</i>	1.21[1.04]	0.91[0.96]	0.464
<i>NLRP3</i>	5.89[5.74]	4.95[6.58]	0.724
<i>NLRP12</i>	0.60[0.40]	1.81[3.15]	0.295
High versus low grade CMV viremia/no viremia			
	CMV viremia >500 copies/ml [n=4] Mean relative level [SD]	CMV viremia ≤ 500 copies/ml or no viremia [n=25] Mean relative level [SD]	
<i>CASP1</i>	5.49[7.51]	1.78[1.03]	0.016
<i>ASC</i>	2.55[1.25]	2.24[1.21]	0.637
<i>NLRP1</i>	1.51[1.27]	0.91[0.92]	0.261
<i>NLRP3</i>	5.62[5.41]	5.14[6.50]	0.890
<i>NLRP12</i>	0.71[0.55]	1.60[2.92]	0.554
Patients with prolonged ICU admission who did and did not develop CMV viremia			
	Patients with CMV viremia [n=6] Mean relative level [SD]	Patients without CMV viremia [n=5] Mean relative level [SD]	
<i>CASP1</i>	4.28[6.20]	1.33[0.60]	0.321
<i>ASC</i>	2.285[1.30]	1.68[1.14]	0.439
<i>NLRP1</i>	1.22[1.11]	1.22[1.64]	0.995
<i>NLRP3</i>	4.87[4.68]	7.74[11.71]	0.593
<i>NLRP12</i>	0.63[0.44]	2.67[4.24]	0.267
Patients with new versus old ICU admission ¹			
	In ICU>48h at time of blood culture [n=11] Mean relative level [SD]	In ICU<48 h at time of blood culture [n=18] Mean relative level [SD]	
<i>CASP1</i>	2.94[4.66]	1.90[1.00]	0.367
<i>ASC</i>	2.01[1.21]	2.44[1.98]	0.353
<i>NLRP1</i>	1.21[1.30]	0.85[0.70]	0.330
<i>NLRP3</i>	6.17[8.25]	4.61[4.88]	0.527
<i>NLRP12</i>	1.56[2.90]	1.42[2.69]	0.898

¹ New ICU (in intensive care unit <48h of bacteremia) vs old ICU (>48h prior to bacteremia)

Table 4

Inflammasome levels and outcomes in the study patients

Patients who died versus those who lived			
Inflammasome	Death (n=4) Mean relative level [SD] ¹	Alive (n=25) Mean relative level [SD]	P-value
<i>CASP1</i>	1.65[1.14]	2.39[3.13]	0.647
<i>ASC</i>	2.45[0.73]	2.29[1.27]	0.794
<i>NLRP1</i>	0.90[0.83]	1.00[1.00]	0.871
<i>NLRP3</i>	5.20[4.81]	5.21[6.56]	0.997
<i>NLRP12</i>	1.03[1.73]	1.55[2.87]	0.7
Patients with multi organ system failure/death			
Inflammasome	MOF ² /death (n=13) Mean relative level [SD]	No MOF and alive (n=16) Mean relative level [SD]	P- level
<i>CASP1</i>	2.91[4.19]	1.70[0.61]	0.182
<i>ASC</i>	2.45[1.07]	2.20[1.32]	0.585
<i>NLRP1</i>	1.09[0.91]	1.08[1.05]	0.975
<i>NLRP3</i>	4.91[5.24]	5.44[7.17]	0.825
<i>NLRP12</i>	0.65[0.96]	2.14[3.47]	0.145

¹SD= standard error;²MOF= multi organ failure