Systemic inflammation and protease profile of Afro-Caribbean patients with sepsis

Panid Borhanjoo[1]*, Navneet Singh[1]* (iD), Sridesh Nath[1],
MD Sadakat Chowdhury[2], Carl Swanson[1], Ryan Kaiser[1],
Patrick Geraghty[1,3], Robert F Foronjy[1,3] and Lillian Chow[1]

Abstract

Objectives: Sepsis is one of the leading causes of morbidity and mortality within the healthcare system and remains a diagnostic and therapeutic challenge. A major issue in the diagnosis of sepsis is understanding the pathophysiologic mechanism, which revolves around host immune system activation and dysregulated responses. African Americans are more likely to experience severe sepsis with higher mortality rates compared to the general population. This pilot study characterized multiple inflammatory markers and proteases in plasma of primarily African American and Afro-Caribbean patients with mild sepsis.

Methods: Plasma was collected from 16 healthy controls and 15 subjects presenting with sepsis, on admission, and again upon resolution of the signs of sepsis, defined as a resolution of sepsis criteria. Plasma samples were analyzed for cytokines, chemokines, and proteases using multiplex bead assays.

Results: Elevated levels of granulocyte colony-stimulating factor, interleukin-10, interleukin-15, interleukin-1 receptor antagonist, interleukin-8, interferon gamma-induced protein 10, monocyte chemoattractant protein-1, matrix metallopeptidase 12, and cathepsin S were identified in plasma from sepsis patients on admission compared to control subjects. Interleukin-6, interleukin-8, granulocyte colony-stimulating factor, and cathepsin S were reduced in sepsis patients upon clinical resolution of sepsis.

Conclusion: These findings profile the circulating inflammatory cytokines, chemokines, and proteases in African Americans and Afro-Caribbean patients during sepsis. The role of these targets in sepsis needs addressing in this patient population.

Introduction

Sepsis is characterized by dysregulation of the body's inflammatory response to infection, leading to several hallmark sequelae including peripheral vasodilation and inadequate tissue oxygenation and perfusion.[1,2] In the United States, sepsis is one of the leading causes of morbidity and mortality within our healthcare system.[3,4] Yet despite the burden that sepsis imposes, diagnosing these patients accurately and efficiently remains a persistent challenge. With the current emergence of the COVID-19 pandemic, a notable disparity is emerging with 27% of the deaths in the United States is from an African American (AA) population while AA accounts for only 12.5% of the total population in the United States.[5] Similar disparities are reflected in the number of hospitalizations among AA compared to disease prevalence in multiple communities and this is not only limited to COVID-19. Population-based studies show that AA individuals have higher rates of sepsis, hospitalization

[1]Division of Pulmonary & Critical Care Medicine, Department of Medicine, State University of New York Downstate Medical Center, Brooklyn, NY, USA
[2]State University of New York Downstate School of Medicine, Brooklyn, NY, USA
[3]Department of Cell Biology, State University of New York Downstate Medical Center, Brooklyn, NY, USA

*Both the authors contributed equally.

Corresponding author:
Lillian Chow, State University of New York Downstate Medical Center, 450 Clarkson Avenue, MSC 19, Brooklyn, NY 11203, USA.
Email: Lillian.chow@downstate.edu

mortality, and are more likely to develop sepsis than Caucasians.[6–8] In a large cohort study looking at infection rates and sepsis incidence, people of color were less likely to experience an infection than Caucasians; however, AA were more likely to experience severe sepsis.[9] These disparities might be partially due to the differences in inflammation responses between races. This is notable with AA and Hispanic children having elevated C-reactive protein compared to Caucasians.[10] Equally, a large proportion of immune response genes are regulated by ancestry control. Therefore, immune responses to infections require profiling in different populations.[11]

Our hospital, the State University of New York Downstate Medical Center serves a predominantly AA and Afro-Caribbean population. Considering the higher baseline inflammation markers in AAs and high mortality in AA septic patients,[12] we profiled inflammatory chemokines, cytokines, growth factors, and protease levels in the plasma of patients with early or mild sepsis to those without sepsis. In addition, inflammation was examined in the same sepsis patients at 3.20 ± 2.34 days after standard treatment and resolution of sepsis. We expected to observe systemic inflammation similar to other published studies in AA and Caucasian populations, with reduced inflammation following the clinical resolution of sepsis. Therefore, the main goal of this study was to profile systemic inflammation and proteases in AA sepsis patients during mild sepsis and upon clinical resolution of sepsis.

Methods

Study population

We conducted a pilot study on all admitted patients within our institution's medicine service between February and November 2018, who fulfilled the inclusion criteria. The study was granted IRB approval by our Institutional Review Board and adherence to the Declaration of Helsinki. Within this time period, the majority of patients were admitted with the suspicion of infection to the medicine service, with no patient suffering from trauma. All patients were enrolled within 24 h of emergency department triage, and therefore, no patients were long-term admissions. Fifteen adult patients were selected for our study group, based on 2001 consensus definitions for sepsis.[13] Patients were referred from either the emergency room, the medical intensive care unit (MICU), or the general medicine ward at SUNY Downstate Health Sciences University from February to November 2018. Fifteen control patients were selected from the general ambulatory clinics at the same institution during that time period given that they were adults who did not meet the predetermined definitions for sepsis. Patients were excluded if they had a known history of the following criteria: sickle cell disease, pulmonary artery hypertension, recent packed red blood cell transfusion, acute coronary syndrome, life-threatening

bleeding, hypercapnia, hypothermia, severe acidosis, and significant anemia with hemoglobin <7 mg/dL.

Sepsis patients had peripheral blood taken on presentation (Sepsis A) and again upon resolution of the signs of sepsis (Sepsis B), with a minimal time interval of 24 h between samples, after admission. Inflammation and protease changes upon resolution of the signs of sepsis were the primary outcome of the study. Control patients had a one-time blood draw of the same quantity upon enrollment. Plasma was isolated from the peripheral blood samples and stored at −80°C until bead assays were performed. The clinical characteristics of each patient were collected at the time of enrollment and the collection of the second sepsis sample. Simplified Acute Physiology Score II (SAPS II) and Sepsis-related Organ Failure Assessment (SOFA) scores were calculated for each patient.[14,15] Written informed consent was obtained from all study participants and approved by the institutional review board of the State University of New York Downstate Medical Center.

Chemokine, cytokine, and protease detection

All samples were analyzed for epidermal growth factor (EGF), interferon (IFN)-α2, IFNγ, interleukin-1 receptor antagonist (IL-1RA), interleukin (IL)-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p40, IL-12p70, IL-15, IL-13, IL-17A, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, interferon gamma-induced protein (IP)-10, tumor necrosis factor (TNF)-α, TNF-β, eotaxin, and vascular endothelial growth factor (VEGF) using a human cytokine 29-multiplex panel (Millipore Chemokine and Cytokine Assay, Millipore). The detection threshold ranged from 0.4 to 26.3 pg/mL over all the 29 bead assay targets. Cathepsin S (CTSS) and matrix metallopeptidase 12 (MMP-12) levels were quantified using a human multiplex bead assay (R&D Systems).

Statistics

In this exploratory pilot study, a sample size was estimated from IL-8 plasma responses in sepsis patients with a three-fold concentrations of IL-8 plasma compared to controls[16] and power of 0.80 with an $\alpha = 0.05$, then a population size of at least 15 samples is recommended, using the UBC online sample size calculator[17] based on the normal approximation to the binomial distribution.[18] All statistical analysis was performed using the GraphPad Prism Software (Version 5 for Mac OS X). D'Agostino and Pearson omnibus normality and F-tests were performed on all data sets. Significant outliers were identified using the Grubbs test (also known as extreme studentized deviate method) and excluded, only if the outlier did not change the result outcome or assumption. Data set pairs were compared by Student's t-test (two-tailed) when

Table 1. Control and sepsis group characteristics/demographics.

	Controls, N = 16 (%)	Sepsis, N = 15 (%)	p-value
Average age (years)	49.25 ± 19.81	61.20 ± 18.17	0.091
Gender (% male)	7 (43.75%)	6 (40.00%)	>0.740
Ethnicity			
African Caribbean	5 (31%)	10 (67%)	>0.085
African American	3 (19%)	3 (20%)	>0.978
Caucasian	4 (25%)	0 (0%)	
Asian	1 (6%)	0 (0%)	
Unknown	3 (19%)	2 (13%)	>0.653
BMI	29.76 ± 12.33	27.74 ± 13.23	0.6002
Comorbidities	1.93 ± 1.71	3.20 ± 1.61	**0.0461**
Smoking Hx (%)	6 (40.00%)	4 (26.77%)	0.2467
Asthma Hx (%)	5 (33.00%)	1 (6.67%)	0.0719
COPD Hx (%)	2 (13.33%)	0 (0.00%)	0.1534
GERD Hx (%)	3 (20.00%)	1 (6.67%)	0.2990

BMI: body mass index; COPD: chronic obstructive pulmonary disease; GERD: gastroesophageal reflux disease.
Data are presented as mean ± SD or percentages in parentheses. Data were analyzed by D'Agostino and Pearson omnibus normality test and further analyzed by either Student's *t*-test (two-tailed) or the Mann–Whitney test. *p*-values are shown here with significant differences in bold.

they passed normality testing and by Mann–Whitney test if they did not pass normality testing. Wilcoxon signed-rank tests were performed on data from the same patient samples from separate days. Data are presented as mean value and standard deviation unless otherwise specified. Exact *p*-values are shown.

Results

Patient demographics

The sepsis group (*N*=15) consisted of six males (40%) whereas the control group (*N*=16) included seven males (43.75%; $p \geq 0.9999$) (Table 1). The mean age for the sepsis group was 61.20 ± 18.17 years, versus 49.25 ± 19.81 years in the control group (*p*-value=0.0913). The three most common ethnicities overall were Afro-Caribbean, AAs, and Caucasians, and they composed 31%, 19%, and 25% of the control group and 67%, 20%, and 0% of the sepsis group, respectively (Table 1). The comorbidity index was 3.20 in the sepsis group and 1.93 in the control group (*p*=0.0461). Four patients (26.77%) were smokers in the sepsis group versus six (40%) in the control group. On admission, the sepsis subjects had an average heart rate, blood pressure, temperature, white blood cell (WBC) count, and lactate level of 119 bpm, 131/83 mm Hg, 101.29°F, $14.59 \times 103/\mu L$, and 2.93 mmol/L, respectively (Table 2). Most infections were of pulmonary source (*n*=6, 40%), followed by genitourinary (*n*=4, 26.67%), skin and soft tissue (*n*=2, 13.33%), bacteremia (*n*=2, 13.3%), and intra-abdominal (*n*=1, 6.67%). The

Table 2. Laboratory and clinical findings of sepsis group subjects on admission.

	N = 15
Age (years)	61.20 ± 18.17
Gender (male n, %)	6 (40%)
Ethnicity	
African Caribbean	10 (67%)
African American	3 (20%)
Unknown	2 (13%)
BMI	27.74 ± 13.23
Clinical findings on admission	
Glasgow Coma Scale (GCS)	14.13 ± 2.10
Heart rate (bpm)	119 ± 16.99
Blood pressure (mm Hg)	131/83 ± 32/23
Mean arterial pressure (MAP) (mm Hg)	84.07 ± 13.71
Temperature (F)	101.29 ± 2.11
WBC count (cells/μL)	14.59 ± 4.43
Hemoglobin (g/dL)	11.86 ± 2.04
Platelets × 10³ (per μL)	207.40 ± 72.10
pH	7.38 ± 0.10
HCO_3 (mEq/L)	23.27 ± 7.03
$PvCO_2$ (mm Hg)	44.08 ± 8.95
Lactate (mmol/L)	2.93 ± 1.43
Creatinine (mg/dL)	1.47 ± 1.69
Bilirubin (mg/dL)	0.87 ± 0.69
Source of infection (%)	
Respiratory	6 (40.00%)
Genitourinary tract	4 (26.67%)
Abdominal	1 (6.67%)
Skin and soft tissue	2 (13.33%)
Bacteremia	2 (13.30%)
Time to the collection of the second sample (days)	3.20 ± 2.34
SOFA score	2.27 ± 1.44
SAPS II score	27.93 ± 12.78

BMI: body mass index; WBC: white blood cell.
Data are presented as mean ± SD or percentages in parentheses. Data were analyzed by the D'Agostino and Pearson omnibus normality test and further analyzed by either Student's *t*-test (two-tailed) or Mann–Whitney test.

time interval between Sepsis A and Sepsis B was 3.20 ± 2.34 days apart. An existing clinical scoring system (SAPS II) was used to correlate measured cytokine levels with the severity of sepsis and mortality. The SAPS II and SOFA scores were 27.93 ± 12.78 and 2.27 ± 1.44 on admission, respectively, and were significantly reduced to 21.27 ± 7.32 (*p*=0.0263) and 0.87 ± 1.30 (*p*=0.0004) after clinical resolution of sepsis (Table 3). Bilirubin was 0.87 ± 0.69 mg/dL on admission and 0.62 ± 0.64 mg/dL (*p*=0.0107) after clinical resolution of sepsis which was statistically significant but clinically insignificant. Glasgow Coma Scale (GCS), platelet count, creatinine, and mean arterial pressure (MAP) did not change significantly from admission to after clinical resolution of sepsis (Table 3).

Table 3. Laboratory and clinical findings of sepsis group subjects on admission (Sepsis A) and upon clinical resolution (Sepsis B).

	Sepsis A	Sepsis B	*p*-value
GCS	14.13 ± 2.10	14.73 ± 0.80	0.1077
Platelets × 10³ (per µL)	207.40 ± 72.10	256.70 ± 129.60	0.0620
Creatinine (mg/dL)	1.47 ± 1.69	1.66 ± 3.16	0.6371
Bilirubin (mg/dL)	0.87 ± 0.69	0.62 ± 0.64	**0.0107**
MAP (mm Hg)	84.07 ± 13.71	89.27 ± 10.07	0.1209
SOFA score	2.27 ± 1.44	0.87 ± 1.30	**0.0004**
SAPS II score	27.93 ± 12.78	21.27 ± 7.32	**0.0263**

GCS: Glasgow Coma Scale; MAP: mean arterial pressure.
Sepsis patients had peripheral blood taken on presentation (Sepsis A) and again upon resolution of the signs of sepsis (Sepsis B) with a minimal time interval of 24 h after admission. Data are presented as mean ± SD values. Data were analyzed by the D'Agostino and Pearson omnibus normality test and further analyzed by either Student's *t*-test (two-tailed) or Mann–Whitney test. *p*-values are shown here with significant differences in bold.

Table 4. Significant elevation of various inflammatory markers and proteases in septic patients (Sepsis A) compared to control subjects.

Target	Control	Sepsis A	*p*-value
G-CSF	28.97 ± 22.30	93.62 ± 46.08	**0.0002**
IL-10	6.54 ± 5.58	40.27 ± 21.82	**<0.0001**
IL-15	4.03 ± 1.82	12.31 ± 8.78	**0.0020**
IL-1RA	17.11 ± 9.88	100.80 ± 94.87	**0.0014**
IL-6	N/D	62.43 ± 73.16	–
IL-8	6.01 ± 9.89	23.76 ± 14.44	**0.0009**
IP-10	764.70 ± 548.90	1884.00 ± 1168.00	**0.0023**
MCP-1	424.50 ± 180.70	627.70 ± 303.40	**0.0171**
MMP-12	0.8 ± 0.2	3.9 ± 0.9	**<0.001**
CTSS	5355.40 ± 1901.10	7821.80 ± 3113.20	**0.002**

G-CSF: granulocyte colony-stimulating factor; IL: interleukin; IL-1RA: interleukin-1 receptor antagonist; IP-10: interferon gamma-induced protein 10; MCP-1: monocyte chemoattractant protein-1; MMP-12: matrix metallopeptidase 12; CTSS: cathepsin S.
Data are presented as mean ± SD. Values detected below the threshold limit defined by assay were labeled as not detectable (N/D). Data were analyzed by the D'Agostino and Pearson omnibus normality test and further analyzed by either Student's *t*-test (two-tailed) or Mann–Whitney test. *p*-values are shown here with significant differences in bold.

Plasma cytokines and proteases differ between control and mild sepsis patients

From the 31 cytokines, chemokines, growth factors, and proteases measured in plasma of septic and control subjects, nine were elevated in the sepsis group. In this analysis, levels of G-CSF (*p*=0.0002), IL-10 (*p*=<0.0001), IL-15 (*p*=0.0020), IL-1RA (*p*=0.0014), IL-8 (*p*=0.0009), IP-10 (*p*=0.0023), MCP-1 (*p*=0.0171), MMP-12 (*p*<0.001), and CTSS (*p*=0.002) were all expressed higher in septic patients on admission (Sepsis A) compared to control subjects (Table 4). IL-6 levels in control subjects were below the detection levels of the assays. However, we detectable in all the Sepsis A

samples (Table 4). The remaining targets were not different (see Supplemental Table 1).

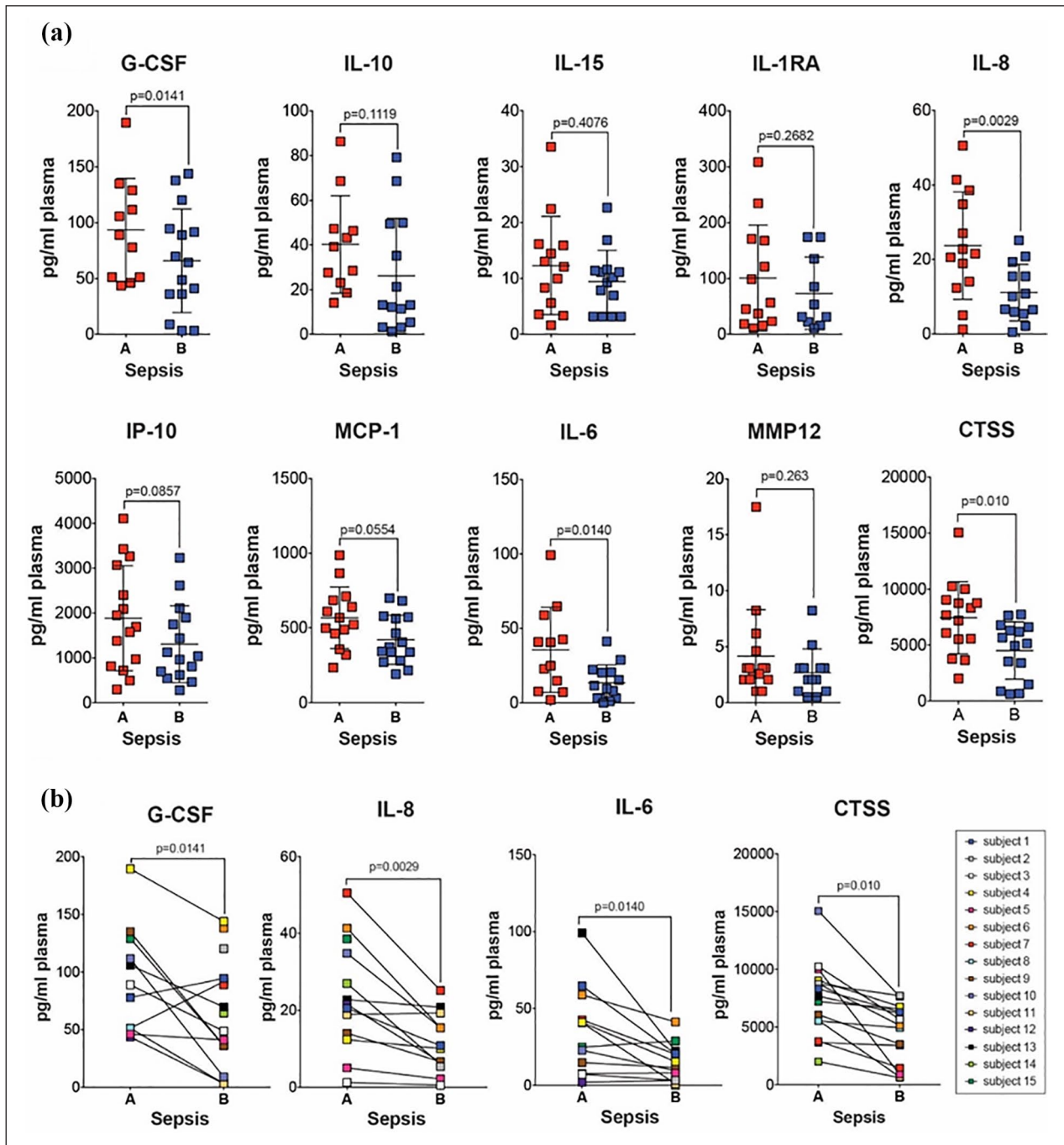### Plasma levels of G-CSF, IL-8, IL-6, and CTSS reduce following clinical resolution

These nine targets that were examined further and re-measured in the septic group upon resolution of sepsis, that is, Sepsis B, using the same assays. In that analysis, levels of G-CSF (*p*=0.0141), IL-6 (*p*=0.0140), IL-8 (*p*=0.0029), and CTSS (*p*=0.010) were reduced in Sepsis B samples compared to Sepsis A (Figure 1).

Discussion

The study profiled various chemokines, cytokines, growth factor, and proteases in the plasma of AA and Afro-Caribbean patients with sepsis to those without sepsis, and further evaluated these changes in the same sepsis patients after the resolution of clinical sepsis symptoms. We further focused this study on patients with early or mild sepsis, paying attention to a population that an Internist or an Emergency Medicine physician will routinely encounter. G-CSF, IL-1RA, IL-8, IL-10, IL-15, IP-10, MCP-1, MMP-12, and CTSS levels were elevated in the plasma of sepsis patients compared to controls. Interestingly, G-CSF, IL-6, IL-8, and CTSS levels were decreased in the plasma of sepsis patients after the resolution of the signs of sepsis. This pilot study gives us important knowledge on inflammation and protease changes in AA and Afro-Caribbean populations. Equally, identifying we are the first to demonstrate altered changes in CTSS in sepsis.

The systemic inflammation status is extensively studied in sepsis. However, some investigators report that most cytokines show similar profiles between patients with mild or severe sepsis.[19] IL-8, MCP-1, and MIP-1β observed in early sepsis and correlate with organ failure.[19] MIP-1β and GM-CSF were also reported to be reduced during sepsis.[19] We were unable to detect MIP-1β in our patient population and only detected GM-CSF in our control patient population. Others report that IL-1β, IL-6, IL-8, IL-12, IFN-γ, G-CSF, and TNF-α remain high in non-survivor sepsis patients.[19] In our study, all patients survived and experienced primarily mild sepsis defined by SOFA and SAPS II scoring. Therefore, it is not surprising that a different cytokine profile is reported. Equally, additional studies observe elevated IL-1β, MCP-1, MIP-1 β, IL-12, IFN-γ, TNF-α,[19] IL-7, IL-10, IL-13,[16] and IL-18.[20] Whether these differences are due to ethnic differences or disease severity need to be addressed. Many studies do not report the ethnic background of the study population. The study by Bozza et al.[16] was performed in Brazil and the study by Rau et al.[20] in Germany do not report the ethnic background of the patient population. Therefore, it is currently difficult to compare our outcomes to other studies.

The targets outlined here that respond upon clinical resolution could play a central role in several responses during

Figure 1. Plasma levels of G-CSF, IL-8, IL-6, and CTSS reduce following clinical resolution. (a) Luminex bead assays were performed for G-CSF, IL-10, IL-15, IL-1RA, IL-8, IP-10, MCP-1, IL-6, MMP-12, and CTSS on plasma from sepsis patients at the time of admission (Sepsis A) and following clinical resolution (Sepsis B). (b) Plasma levels of G-CSF, IL-8, IL-6, and CTSS from Sepsis A and B. Corresponding squares connected by a line to demonstrate trend for individual subjects. Concentrations are given in pg/mL of plasma. Data are presented as mean values and SD. Data were analyzed by D'Agostino and Pearson omnibus normality test and further analyzed by the Wilcoxon signed-rank test. *p*-values are shown comparing groups connected by a line.

sepsis. IL-6, produced by multiple cell types, has both anti-inflammatory and pro-inflammatory roles depending on the signaling pathway.[21] The severity of sepsis correlates with the early levels of IL-6.[22] In our study population, IL-6 levels were not detectable in controls, unlike septic patient samples. IL-6 is reported to be associated with the highest risk of death in patients with sepsis.[23] Similar to IL-6, elevated IL-8 is frequently observed in sepsis patients.[24,25] IL-8 is produced by multiple cell types, including macrophages, and serves to attract neutrophils to the site of infection.[26]

Multiple studies suggest that IL-8 may have a unique role in the immune response to sepsis. A study in burn patients showed that high IL-8 levels correlated with increased risk for multi-organ failure, sepsis, and mortality.[27] Likewise, researchers found increased IL-6, IL-8, and MCP-1 plasma levels in septic patients who died compared to survivors.[28] Increased IL-8 levels in sepsis were also associated with the risk of developing acute lung injury.[29] In a Brazilian study, IL-8 and MCP-1 exhibited the best correlation with the SOFA score.[16] In agreement with the preceding studies, our study found that plasma IL-8 levels coincided with SAPS II and SOFA scores in AAs. In fact, IL-8 demonstrated the greatest reduction in levels in the follow-up sepsis samples ($p < 0.003$). Furthermore, IL-8 plasma levels declined by 80% (12 patients) of the sepsis cohort. A potential limitation in the utility of IL-8 as a clinical readout of disease severity is its lack of specificity for diagnosing sepsis, as it is elevated in many other inflammatory disease states[30] and may lack the diagnostic power that is needed in the identification of patients with sepsis. When compared to procalcitonin, a commonly utilized marker, IL-8 does not demonstrate the same level of clinical utility.[31] This uncertainty in the literature suggests that more research is required to establish the role of IL-8 in response to sepsis.

We also observed changes in G-CSF in our study population. G-CSF is a hematopoietic cell stimulator released by macrophages, epithelial cells, and T cells, which promotes cellular proliferation and differentiation and neutrophil migration and cytokine release.[32] In contrast to IL-8, G-CSF is believed to play a protective role in sepsis and researchers have investigated its use in infectious disease states like pneumonia.[33,34] A study of nearly 760 pneumonia subjects found that G-CSF administration did not improve mortality or length of stay. It did, however, increase neutrophil numbers and reduced the incidence of complications like acute respiratory distress syndrome (ARDS) and empyema.[33] In agreement with our study, another group found that G-CSF levels were the highest in early sepsis (within 3 h of diagnosis) and remained persistently elevated during the course of the disease.[35] Interestingly, pretreatment with G-CSF improved survival in murine models of peritonitis and sepsis.[36,37] Given its role in neutrophil maintenance and cellular proliferation, it is conceivable that G-CSF could be exerting protective effects on immune responses and tissue repair in sepsis.

To our knowledge, this is the first report demonstrating that CTSS levels changes in sepsis. CTSS is a protein found inside the lysosomal/endosomal compartments of primarily antigen-presenting cells and plays a significant role in various intracellular and extracellular processes, including proteolysis and major histocompatibility complex (MHC) class II-mediated immune responses.[38] It has a recognized role in several different disease processes including various pulmonary diseases, cardiovascular disease, diabetes, and cancer;[39–42] however, its role in sepsis is unclear. CTSS may play a role in sepsis by inducing apoptosis by causing direct mitochondrial damage and mediating cytochrome c release.[43] Importantly, recent data suggest that circulating CTSS protein levels are associated with increased mortality risk in elderly men and women,[44] and CTSS is associated with the promotion of pain signaling.[45,46]

Like CTSS, MMP-12 is a protease that degrades elastase and is primarily expressed in macrophages and plays a significant role in several diseases.[47] In addition, MMP-12 could play a role in sepsis as MMP inhibitors prevent sepsis-induced refractoriness to vasoconstrictors in rat sepsis models.[48] The MMP-12 protein can directly induce an early inflammatory response characterized by neutrophil infiltration, cytokine release, gelatinase activation, and delayed macrophage recruitment.[49] More research is needed to further understand the functional role of proteases in sepsis.

Despite multiple important observations from our study, there are several limitations that we need to discuss. There were only 15–16 subjects in each arm of the study. Increased sample size and study power are required to verify the significance of our data. Of note, a recent study found that plasma MCP-1, soluble CD14, IL-6, and IL-10 levels were higher in subjects with pneumonia and infective endocarditis compared to those with bacterial meningitis.[50] While we cataloged the source of infection in our study, we have not discussed any possible associations due to the small cohort size. It is also conceivable, that larger sample size may have detected changes in other plasma cytokine/chemokines. It is important to note that our study may not be generalizable to all sepsis patients. Our study focuses on patients with early or mild sepsis and all patients survived. More appreciable differences in these cytokines/chemokines may be identified with larger sample size and including patients presenting with higher initial SAPS II and SOFA scores correlating with a more severe clinical presentation. Equally, although our patient population is primarily AA and Afro-Caribbean and we collected samples from sepsis and control subjects within our institution, several patients were not AA or Afro-Caribbean. Additional studies are needed comparing larger cohorts from multiple ethnicities. Our sepsis cohort also has higher numbers of comorbidities than the control group. Finally, all our study subjects were adults so it is uncertain whether these findings would apply to a pediatric population.

Conclusion

Our observations suggest that further investigations into the roles of IL-6, IL-8, G-CSF, and CTSS in sepsis in the Afro-Caribbean and AA population. Equally, they may represent good indicators for sepsis resolution. Further work is necessary to confirm the significance of these inflammatory proteins in sepsis, compare the expression and circulating levels of inflammatory proteins among ethnic subpopulations, investigate if they can be used in the diagnosis of sepsis and monitoring response to therapeutic interventions, and their

use to improve treatment of sepsis. Finally, further studies are required on the role of CTSS in sepsis, as this is the first time CTSS is reported to be elevated in sepsis.

Declaration of conflicting interests

Ethical approval

Ethical approval for this study was obtained from SUNY Downstate Medical Center IRB (901180-2).

Funding

Informed consent

Written informed consent was obtained from all subjects before the study.

ORCID iD

Navneet Singh 🔟 https://orcid.org/0000-0001-8058-3004

Supplemental material

Supplemental material for this article is available online.

References

1. Pinsky MR. Dysregulation of the immune response in severe sepsis. *Am J Med Sci* 2004; 328(4): 220–229.
2. Lush CW and Kvietys PR. Microvascular dysfunction in sepsis. *Microcirculation* 2000; 7: 83–101.
3. Vincent JL, Marshall JC, Namendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *Lancet Respir Med* 2014; 2(5): 380–386.
4. Fleischmann C, Scherag A, Adhikari NK, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am J Respir Crit Care Med* 2016; 193: 259–272.
5. Azar KMJ, Shen Z, Romanelli RJ, et al. Disparities in outcomes among COVID-19 patients in a large health care system in California. *Health Aff* 2020; 39: 1253–1262.
6. Barnato AE, Alexander SL, Linde-Zwirble WT, et al. Racial variation in the incidence, care, and outcomes of severe sepsis: analysis of population, patient, and hospital characteristics. *Am J Respir Crit Care Med* 2008; 177: 279–284.
7. Dombrovskiy VY, Martin AA, Sunderram J, et al. Occurrence and outcomes of sepsis: influence of race. *Crit Care Med* 2007; 35(3): 763–768.
8. Martin GS, Mannino DM, Eaton S, et al. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546–1554.
9. Moore JX, Donnelly JP, Griffin R, et al. Black-white racial disparities in sepsis: a prospective analysis of the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. *Crit Care* 2015; 19: 279.
10. Schmeer KK and Tarrence J. Racial-ethnic disparities in inflammation: evidence of weathering in childhood. *J Health Soc Behav* 2018; 59(3): 411–428.
11. Nedelec Y, Sanz J, Baharian G, et al. Genetic ancestry and natural selection drive population differences in immune responses to pathogens. *Cell* 2016; 167: 657–669.e21.
12. Matter ML, Shvetsov YB, Dugay C, et al. High mortality due to sepsis in Native Hawaiians and African Americans: the multiethnic cohort. *PLoS ONE* 2017; 12(5): e0178374.
13. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ ACCP/ATS/SIS international sepsis definitions conference. *Intensive Care Med* 2003; 29: 530–538.
14. Le Gall JR, Lemeshow S and Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA* 1993; 270: 2957–2963.
15. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med* 1996; 22(7): 707–710.
16. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Crit Care* 2007; 11(2): R49.
17. Brant R. Inference for Means: Comparing Two Independent Samples, https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html
18. Rosner B. *Fundamentals of Biostatistics*. Boston, MA: Cengage Learning, 2010.
19. Mera S, Tatulescu D, Cismaru C, et al. Multiplex cytokine profiling in patients with sepsis. *APMIS* 2011; 119(2): 155–163.
20. Rau M, Schiller M, Krienke S, et al. Clinical manifestations but not cytokine profiles differentiate adult-onset Still's disease and sepsis. *J Rheumatol* 2010; 37: 2369–2376.
21. Scheller J, Chalaris A, Schmidt-Arras D, et al. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 2011; 1813(5): 878–888.
22. Novotny AR, Reim D, Assfalg V, et al. Mixed antagonist response and sepsis severity-dependent dysbalance of pro- and anti-inflammatory responses at the onset of postoperative sepsis. *Immunobiology* 2012; 217(6): 616–621.
23. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med* 2007; 167: 1655–1663.
24. Hack CE, Hart M, van Schijndel RJ, et al. Interleukin-8 in sepsis: relation to shock and inflammatory mediators. *Infect Immun* 1992; 60(7): 2835–2842.
25. Van Zee KJ, DeForge LE, Fischer E, et al. IL-8 in septic shock, endotoxemia, and after IL-1 administration. *J Immunol* 1991; 146: 3478–3482.
26. Turner MD, Nedjai B, Hurst T, et al. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta* 2014; 1843(11): 2563–2582.
27. Kraft R, Herndon DN, Finnerty CC, et al. Predictive value of IL-8 for sepsis and severe infections after burn injury: a clinical study. *Shock* 2015; 43(3): 222–227.
28. Hong TH, Chang CH, Ko WJ, et al. Biomarkers of early sepsis may be correlated with outcome. *J Transl Med* 2014; 12: 146.
29. Liu XW, Ma T, Cai Q, et al. Elevation of serum PARK7 and IL-8 levels is associated with acute lung injury in patients with severe sepsis/septic shock. *J Intensive Care Med* 2019; 34(8): 662–668.

30. Harada A, Sekido N, Akahoshi T, et al. Essential involvement of interleukin-8 (IL-8) in acute inflammation. *J Leukoc Biol* 1994; 56(5): 559–564.

31. Harbarth S, Holeckova K, Froidevaux C, et al. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. *Am J Respir Crit Care Med* 2001; 164: 396–402.

32. Eyles JL, Hickey MJ, Norman MU, et al. A key role for G-CSF-induced neutrophil production and trafficking during inflammatory arthritis. *Blood* 2008; 112: 5193–5201.

33. Nelson S, Belknap SM, Carlson RW, et al. A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. *J Infect Dis* 1998; 178(4): 1075–1080.

34. Root RK, Lodato RF, Patrick W, et al. Multicenter, double-blind, placebo-controlled study of the use of filgrastim in patients hospitalized with pneumonia and severe sepsis. *Crit Care Med* 2003; 31(2): 367–373.

35. Tanaka H, Ishikawa K, Nishino M, et al. Changes in granulocyte colony-stimulating factor concentration in patients with trauma and sepsis. *J Trauma* 1996; 40(5): 718–725; discussion725.

36. O'Reilly M, Silver GM, Greenhalgh DG, et al. Treatment of intra-abdominal infection with granulocyte colony-stimulating factor. *J Trauma* 1992; 33(5): 679–682.

37. Villa P, Shaklee CL, Meazza C, et al. Granulocyte colony-stimulating factor and antibiotics in the prophylaxis of a murine model of polymicrobial peritonitis and sepsis. *J Infect Dis* 1998; 178(2): 471–477.

38. Wilkinson RD, Williams R, Scott CJ, et al. Cathepsin S: therapeutic, diagnostic, and prognostic potential. *Biol Chem* 2015; 396(8): 867–882.

39. Chapman HA, Riese RJ and Shi GP. Emerging roles for cysteine proteases in human biology. *Annu Rev Physiol* 1997; 59: 63–88.

40. Sena BF, Figueiredo JL and Aikawa E. Cathepsin S as an inhibitor of cardiovascular inflammation and calcification in chronic kidney disease. *Front Cardiovasc Med* 2017; 4: 88.

41. Jobs E, Risérus U, Ingelsson E, et al. Serum cathepsin S is associated with decreased insulin sensitivity and the development of type 2 diabetes in a community-based cohort of elderly men. *Diabetes Care* 2013; 36(1): 163–165.

42. Zhang L, Wang H and Xu J. Cathepsin S as a cancer target. *Neoplasma* 2015; 62: 16–26.

43. Pinheiro da Silva F and Nizet V. Cell death during sepsis: integration of disintegration in the inflammatory response to overwhelming infection. *Apoptosis* 2009; 14(4): 509–521.

44. Jobs E, Ingelsson E, Riserus U, et al. Association between serum cathepsin S and mortality in older adults. *JAMA* 2011; 306: 1113–1121.

45. Clark AK, Yip PK, Grist J, et al. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proc Natl Acad Sci U S A* 2007; 104: 10655–10660.

46. Clark AK, Yip PK and Malcangio M. The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. *J Neurosci* 2009; 29: 6945–6954.

47. Qin Y, Cao X, Yang Y, et al. Cysteine protease cathepsins and matrix metalloproteinases in the development of abdominal aortic aneurysms. *Future Cardiol* 2013; 9(1): 89–103.

48. de Souza P, Schulz R and da Silva-Santos JE. Matrix metalloproteinase inhibitors prevent sepsis-induced refractoriness to vasoconstrictors in the cecal ligation and puncture model in rats. *Eur J Pharmacol* 2015; 765: 164–170.

49. Nenan S, Boichot E, Planquois JM, et al. Effects of depletion of neutrophils or macrophages on the inflammatory response induced by metalloelastase (MMP-12) in mice airways. *Eur J Pharmacol* 2008; 579: 374–381.

50. Holub M, Dzupova O, Ruzkova M, et al. Selected Biomarkers Correlate with the Origin and Severity of Sepsis. *Mediators Inflamm* 2018; 2018: 7028267.