Three Hypothetical Inflammation Pathobiology Phenotypes and Pediatric Sepsis-Induced Multiple Organ Failure Outcome*

Joseph A. Carcillo, MD[1]; E. Scott Halstead, MD[1]; Mark W. Hall, MD[2]; Trung C. Nguyen, MD[3]; Ron Reeder, PhD[4]; Rajesh Aneja, MD[1]; Bita Shakoory, MD[5]; Dennis Simon, MD[1]; on behalf of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network Investigators

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[1]Department of Critical Care Medicine, Children's Hospital of Pittsburgh and University of Pittsburgh, Pittsburgh, PA.

[2]Department of Pediatrics, Nationwide Children's Hospital and Ohio State University, Columbus, OH.

[3]Department of Pediatrics, Texas Children's Hospital and Baylor College of Medicine, Houston, TX.

[4]Department of Pediatrics, University of Utah, Salt Lake City, UT.

[5]Department of Medicine, George Washington University Hospital, Washington, DC.

Additional members of *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network investigators are as follows: Jeri Burr, MS, RN-BC, CCRN (University of Utah and Department of Pediatrics); Allan Doctor, MD (St. Louis Children's Hospital and Washington University Department of Pediatrics); Christopher J. L. Newth, MD, FRCPC (Children's Hospital Los Angeles and University of Southern California Department of Pediatrics); David L. Wessel, MD (Children's National Medical Center and George Washington University Department of Pediatrics); Kathleen L. Meert, MD (Children's Hospital of Michigan and Wayne State University Department of Pediatrics); J. Michael Dean, MD (University of Utah and Department of Pediatrics); Murray Pollack, MD (Phoenix Children's Hospital); Robert A. Berg, MD (Children's Hospital of Philadelphia and University of Pennsylvania Department of Anesthesiology); Thomas Shanley, MD (C. S. Mott Children's Hospital and University of Michigan Department of Pediatrics); Rick Harrison, MD (Mattel Children's Hospital and University of California Los Angeles Department of Pediatrics); Richard Holubkov, PhD (University of Utah and Department of Pediatrics); Tammara L. Jenkins, MSN, RN, PCNS-BC, and Robert F. Tamburro, MD (Eunice Kennedy Shriver National Institute of Child Health and Human Development).

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Address requests for reprints to: Joseph A. Carcillo, MD, Division of Pediatric Critical Care Medicine, Faculty Pavilion, Children's Hospital of Pittsburgh, 4400 Penn Avenue, Pittsburgh, PA. E-mail: carcilloja@ccm.upmc.edu

Objectives: We hypothesize that three inflammation pathobiology phenotypes are associated with increased inflammation, proclivity to develop features of macrophage activation syndrome, and multiple organ failure-related death in pediatric severe sepsis.

Design: Prospective cohort study comparing children with severe sepsis and any of three phenotypes: 1) immunoparalysis-associated multiple organ failure (whole blood ex vivo tumor necrosis factor response to endotoxin < 200 pg/mL), 2) thrombocytopenia-associated multiple organ failure (new onset thrombocytopenia with acute kidney injury and a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 activity < 57%), and/or 3) sequential multiple organ failure with hepatobiliary dysfunction (respiratory distress followed by liver dysfunction with soluble Fas ligand > 200 pg/mL), to those without any of these phenotypes.

Setting: Tertiary children's hospital PICU.

Patients: One hundred consecutive severe sepsis admissions.

Interventions: Clinical data were recorded daily, and blood was collected twice weekly.

Measurements and Main Results: Multiple organ failure developed in 75 cases and eight died. Multiple organ failure cases with any of the three inflammation phenotypes ($n = 37$) had higher inflammation (*C*-reactive protein, $p = 0.009$ and ferritin, $p < 0.001$) than multiple organ failure cases without any of these phenotypes ($n = 38$) or cases with only single organ failure ($n = 25$). Development of features of macrophage activation syndrome and death

were more common among multiple organ failure cases with any of the phenotypes (macrophage activation syndrome: 10/37, 27%; death: 8/37, 22%) compared to multiple organ failure cases without any phenotype (macrophage activation syndrome: 1/38, 3%; $p = 0.003$ and death: 0/38, 0%; $p = 0.002$).

Conclusions: Our approach to phenotype categorization remains hypothetical, and the phenotypes identified need to be confirmed in multicenter studies of pediatric multiple organ dysfunction syndrome. (*Pediatr Crit Care Med* 2017; 18:513–523)

Key Words: immunoparalysis; macrophage activation syndrome; pediatric sepsis; sequential multiple organ failure; thrombocytopenia-associated multiple organ failure

Severe sepsis remains a leading cause of death among children worldwide. Most children dying from sepsis in the resource rich setting do so with multiple organ failure (MOF) (1, 2). Present-day treatment is directed to removing the infection source and supporting organ dysfunction without addressing inflammation pathobiology. Nevertheless, three inflammation phenotypes related to abnormal immune and coagulation responses have been reported to respond to pathobiology targeted therapies (3–18). These inflammation pathobiology-based phenotypes include 1) immunoparalysis-associated MOF (3–8), 2) thrombocytopenia-associated MOF (TAMOF) (9–13), and 3) sequential MOF (SMOF) with new hepatobiliary dysfunction (14–18).

Children with "immunoparalysis syndrome" have lymphoid organ depletion, prolonged reduction in innate and adaptive immune function, and an inability to clear bacterial or fungal infections. These children can be identified by a decreased ex vivo whole blood tumor necrosis factor alpha (TNF-α response to endotoxin, decreased monocyte human leukocyte antigen-antigen D related (HLA-DR) expression, or lymphopenia for more than 3 days (3, 4). Immune function can be restored with immunomodulation or immunosuppressant tapering (4, 7, 19–27). Hyperinflammation in this phenotype has been related to persistent infection and a decreased monocyte TNF response to endotoxin accompanied by an increased systemic interleukin (IL)-6 and IL-10 response. Reversal of immunoparalysis with immunosuppressant tapering or granulocyte macrophage-colony stimulating factor (GM-CSF) therapy restores the monocyte TNF response to endotoxin while decreasing systemic inflammation measured by IL-6 and IL-10 levels (4).

Children with "TAMOF" have reduced ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity that contributes to inability to resolve von Willebrand Factor: platelet clots and ensuing thrombotic microangiopathy (TMA), which can be reversed with plasma exchange therapy (9, 13, 28). Hyperinflammation in these children has been associated with complement overactivation in thrombotic thrombocytopenic purpura (TTP)/atypical hemolytic uremic syndrome (aHUS) as well as necrosis related to microvascular thrombosis in disseminated intravascular coagulation (DIC). Eculizumab (C5a antibody) is a Food and Drug Administration (FDA) approved drug for aHUS that reverses hypercomplementemia-driven inflammation (29).

Children with "SMOF" with new hepatobiliary dysfunction (respiratory followed several days later by liver dysfunction) have a proclivity to natural killer/cytotoxic T lymphocyte dysfunction with an inability to induce death of viruses, cancer cells, or activated immune cells. Viral infections in these children cause lymphoproliferation with soluble Fas ligand (sFasL) release, hemophagocytosis, and sFasL-mediated liver injury which can respond to varied treatment strategies (14–19, 30, 31). Hyperinflammation in these children can be related to their inability to clear viral infections or to induce apoptosis of activated immune cells. Rituximab (CD20 antibody) is FDA approved to reverse posttransplantation Epstein-Barr virus-related lymphoproliferative disease (PTLD) and to kill the virus by removing its reservoir.

The final common pathway of uncontrolled inflammation manifests as the "macrophage activation syndrome" (MAS). Ravelli et al (32) define MAS in children with uncontrolled systemic juvenile idiopathic arthritis by the development of a unique "hyperinflammation" MOF organ failure pattern which includes new onset hepatobiliary dysfunction, DIC, and hyperferritinemia. When recognized early, these children respond well to anti-inflammatory strategies including IL-1 receptor antagonist protein (IL-1ra; anakinra). We recently applied these clinical criteria post hoc to adults with severe sepsis who had been enrolled in an IL-1 receptor blockade trial (33). Overall, 5.6% of the adults in this severe sepsis trial had features of MAS. Among these subjects, anakinra increased survival two-fold from 34.3% with placebo to 65.4% with IL-1 blockade, whereas anakinra had no effect on outcome in severe sepsis subjects without these three combined features of MAS (33).

Adult patients are being actively recruited to participate in nine ongoing clinical trials of specific therapies targeting these three inflammation pathobiology phenotypes, and both adult and pediatric patients are being recruited into two additional trials targeting uncontrolled inflammation and MAS (**Supplementary Table 1**, Supplemental Digital Content 1, http://links.lww.com/PCC/A402). Before garnering any enthusiasm for investigating these personalized inflammation pathobiology-based therapeutic approaches to improve MOF outcomes in pediatric sepsis, it is necessary to first assess whether these inflammation pathobiology phenotypes are associated with adverse outcomes in children with severe sepsis. We test the hypothesis that children with one or more of these inflammation pathobiology-based phenotypes have more inflammation (indirectly indicated by higher *C*-reactive protein [CRP] and ferritin levels), with a greater proclivity to development of features of MAS, and a greater risk of MOF-related death.

MATERIALS AND METHODS

The study was approved by the University of Pittsburgh—Children's Hospital of Pittsburgh and University of Pittsburgh Medical Center (UPMC) Institutional Review Board. Patients were recruited and enrolled after obtaining informed consent from parent(s) at the University of Pittsburgh—Children's

Hospital of Pittsburgh of UPMC—one site of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (NICHD-CPCCRN). The study was planned, performed, and analyzed during the second NICHD-CPCCRN cycle between the years 2009 and 2014. Inclusion criteria were diagnosis of severe sepsis (sepsis with at least one organ failure), an existing indwelling central venous and/or arterial catheters for blood draws, age greater than or equal to 44-week gestation and less than 18 years, and commitment to aggressive care.

The first 2 mL blood sample was obtained on the second day of severe sepsis from an indwelling arterial or central venous catheter at that time, and then twice weekly as long as the catheter was in place, to a maximum of 28 days in the PICU (maximum eight samples for batch analysis). Lipopolysaccharide (LPS)-induced TNF-α production capacity was measured as previously described (4). The remaining whole blood was spun down and the plasma was separated into aliquots and frozen at –80°C for later batch analysis. The TNF-α and sFasL assays were performed using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). ADAMTS13 activity was measured by a commercial assay kit (Immucor GTI Diagnostics/Lifecodes, Waukesha, WI; Immucor.com). Ferritin and CRP were measured in the Children's Hospital of Pittsburgh-UPMC clinical laboratory using the same standard operating procedure used for clinical specimens in our hospitals. The peak Ferritin and CRP sample levels from the total number of samples were used for data analysis.

"Sepsis" was defined by the presence of two or more of the following four criteria: 1) tachycardia (heart rate > 90th percentile for age in absence of stimulation), 2) tachypnea (respiratory rate > 90th percentile for age), 3) abnormal temperature (< 36°C or > 38.5°C), and 4) abnormal WBC count (> 12,000 mm^3 or < 4,000 mm^3 or > 10% immature neutrophils), plus suspicion of infection (www.mdcalc.com/pediatric-sirs-sepsis-criteria). "Organ failure" was defined using the organ failure index (OFI) criteria established by Doughty et al (14) (cardiovascular—need for cardiovascular agent infusion support; pulmonary—need for mechanical ventilation support with the ratio of the Pao_2/Fio_2 < 300 without this support; hepatic—total bilirubin > 1.0 mg/dL and alanine aminotransferase > 100 U/L; renal—serum creatinine > 1.0 mg/dL and oliguria [urine output < 0.5 mL/kg/hr]; hematologic—thrombocytopenia < 100,000/mm^3 and prothrombin time international normalized ratio > 1.5 × normal; and CNS—Glasgow Coma Scale < 12 in absence of sedatives). "Severe sepsis" was defined by the presence of sepsis and one or more organ failures. "MOF" was defined by the development of two or more organ failures. "Immunoparalysis-associated MOF" (4), "TAMOF (9), "SMOF with new hepatobiliary dysfunction" (14), and "MAS" (32, 33) were defined by criteria in **Table 1**. Mortality was defined by death in the PICU (Table 1).

Associations between each inflammation phenotype and admission epidemiology characteristics (age, gender, chronic illness, cancer status, transplantation status) and infection status (bacterial, viral, fungal, culture negative) were evaluated using Fisher exact test or two-sided Wilcoxon rank-sum with normal approximation and continuity correction. The Wilcoxon-Mann-Whitney rank-sum test with two-sided alternative was used to compare CRP and ferritin concentrations between groups. Fisher exact test was used to compare the development of MAS and mortality between groups. A p value of less than 0.05 was considered statistically significant. A secondary analysis was performed excluding the four patients who were admitted twice in the study.

TABLE 1. Clinical and Biomarker Criteria Used to Define Inflammation Phenotypes and Macrophage Activation Syndrome

Group	Clinical Criteria	Confirmatory Biomarker
Immunoparalysis-associated MOF (immune depression)	1) Beyond third day of critical illness with MOF	Whole blood ex vivo TNF response to lipopolysaccharide < 200 pg/mL
Thrombocytopenia-associated MOF (thrombotic microangiopathy)	1) New thrombocytopenia < 100,000, or if baseline platelet count < 100,000 then 50% decrease from baseline 2) Elevated lactate dehydrogenase > 250 U/L 3) Creatinine > 1 mg/dL and oliguria	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 activity < 57% of control
Sequential MOF with new hepatobiliary dysfunction (virus/lymphoproliferative disease disease)	1) Pao_2/Fio_2 < 300 with need for mechanical ventilation 2) Followed days later by new onset hepatic dysfunction ALT > 100 U/L + Bilirubin > 1 mg/dL	Soluble Fas ligand > 200 pg/mL
Macrophage activation syndrome (hyperinflammation common end pathway)	1) Disseminated intravascular coagulation with platelet count < 100,000 and international normalized ratio > 1.5 2) New hepatic dysfunction ALT > 100 U/L + bilirubin > 1 mg/dL	Ferritin > 500 ng/mL

ALT = alanine aminotransferase, MOF = multiple organ failure.

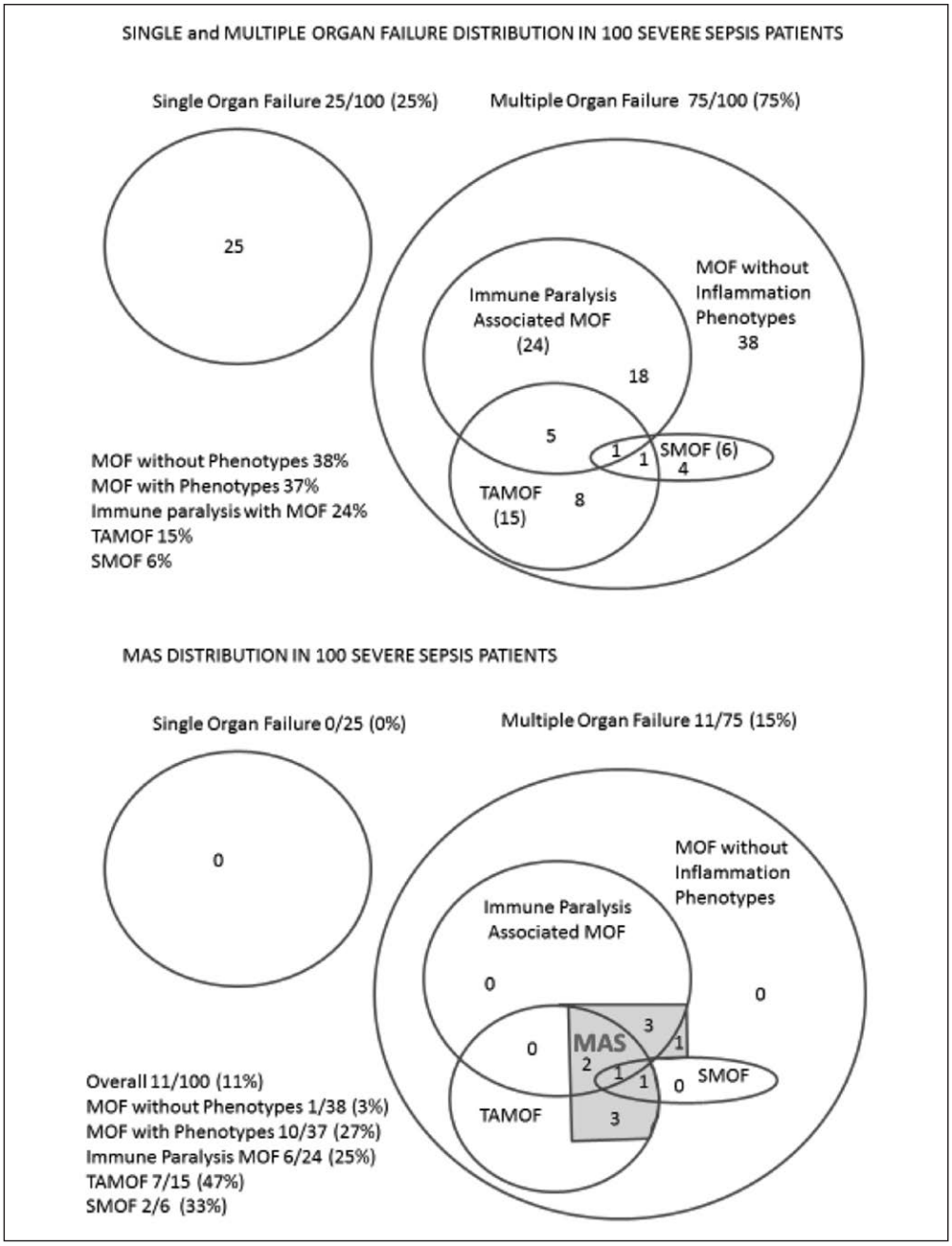

Figure 1. Distribution of single organ failure, multiple organ failure with and without inflammation phenotypes, and macrophage activation syndrome in the severe sepsis population: Nearly one half of multiple organ failure (MOF) patients had one or more of the three inflammation phenotypes (*top*). Macrophage activation syndrome (MAS) was more commonly found in MOF patients with the inflammation phenotypes (10/37) than MOF patients without any of the inflammation phenotypes (1/38); Fisher exact test $p = 0.003$ (*bottom*). SMOF = sequential MOF, TAMOF = thrombocytopenia-associated MOF.

RESULTS

Ninety-six patients comprised 100 consecutive PICU severe sepsis cases. The average age of the children was 5.8 years (SD = 5.7). There were 47 female and 53 male cases. Fifty-nine percent of cases had a chronic illness (14 cancer, 25 solid organ or hematopoietic transplant, 20 other) and 41% were previously healthy. Seventy-five of the cases were culture positive (57 bacterial, 23 viral, nine fungal) and 25 were culture negative. The average Pediatric Risk of Mortality (PRISM) score was 10.7 (SD = 8.7). The average number of maximum organ failures was 2.4 (SD = 1.3). The average number of blood sampling study days was 10.2 (SD = 7.9) with an average of three blood samples attained per case.

Among the 100 cases of severe sepsis, single organ failure was noted in 25 patients, MOF developed in 38 without any of the three inflammation phenotypes (mean OFI = 2.3, SD = 0.5), and MOF developed in 37 with one or more inflammation phenotypes (mean OFI = 3.4, SD = 1.4). In the 37 cases with MOF and one or more inflammation phenotypes, the distribution was 24 of 37 immunoparalysis-associated MOF, 15 of 37 TAMOF, and six of 37 SMOF (**Fig. 1**, *top*). Thirty of these 37 had one distinct phenotype, six of 37 had two phenotypes overlapping, and one of 37 had three phenotypes overlapping.

In univariate analysis, the only significant associations between any of the three inflammation phenotypes and age, gender, chronic illness, cancer, transplantation, or infection type status were found in the immunoparalysis-associated MOF phenotype (**Table 2**). Immunoparalysis-associated MOF was more likely to be observed in cases with increased age (yr: 7.8 ± 5.97 vs 5.2 ± 5.49 mean $\pm$ SD; $p < 0.05$), chronic illness (20/59; 34% with chronic illness vs 4/41; 10% without; $p < 0.05$), or cancer (10/14; 71% with cancer vs 14/86; 16% without; $p < 0.05$).

Compared with absolute lymphocyte count (ALC) less than 1,000/μL, a low ex vivo TNF production was associated with longer length of stay (length of stay—ALC < 1,000: coefficient = –1.5211, SE = 1.8112, $t = -0.84$, $p = 0.403$, 95% CI = –5.1160 to 2.07367; low ex vivo TNF—coefficient = 5.9973, SE = 1.9814, $t = 3.03$, $p = 0.03$, 95% CI = 2.0646–9.9301) and a nonsignificant tendency toward increased mortality (mortality—ALC < 1,000: coefficient = 0.5888, SE = 0.8841, $z = 0.67$, $p = 0.505$, 95% CI = –1.1441 to 2.3218; low ex vivo

TABLE 2. Baseline Characteristics of Each Inflammation Phenotype

	Immunoparalysis-Associated MOF		Thrombocytopenia-Associated MOF		Sequential MOF/ Hepatobiliary Dysfunction	
Baseline Status	**No (*n* = 76)**	**Yes (*n* = 24)**	**No (*n* = 85)**	**Yes (*n* = 15)**	**No (*n* = 94)**	**Yes (*n* = 96)**
Age, mean (SD)	5.2 (5.49)	7.8 (5.97)[a]	5.5 (5.66)	7.7 (5.73)	5.9 (5.82)	4.2 (3.02)
Gender (%)						
Female	38 (50.0)	9 (38)	40 (47)	7 (47)	45 (48)	2 (33)
Male	38 (50.0)	15 (62)	45 (53)	8 (53)	49 (52)	4 (67)
Bacteria (%)						
No	34 (45)	9 (38)	38 (45)	5 (33)	40 (43)	3 (50)
Yes	42 (55)	15 (62)	57 (55)	10 (67)	54 (57)	3 (50)
Virus (%)						
No	58 (76)	19 (79)	65 (77)	12 (80)	72 (77)	5 (83)
Yes	18 (24)	5 (21)	20 (24)	3 (20)	22 (23)	1 (17)
Fungus (%)						
No	69 (91)	22 (92)	76 (89)	15 (100)	86 (92)	5 (83)
Yes	7 (9)	2 (8)	9 (11)	0 (0)	8 (8)	1 (17)
Chronic illness at admission (%)						
No	37 (49)	4 (17)	37 (44)	4 (27)	39 (42)	2 (33)
Yes	39 (51)	20 (83)[b]	48 (56)	11 (73)	55 (59)	4 (67)
Cancer (%)						
No	72 (95)	14 (58)	73 (86)	13 (87)	81 (86)	5 (83)
Yes	4 (5)	10 (42)[c]	12 (14)	2 (13)	13 (14)	1 (17)
Transplant (%)						
No	59 (78)	16 (67)	64 (75)	11 (73)	70 (75)	5 (83)
Yes	17 (22)	8 (33)	21 (25)	4 (27)	24 (26)	1 (17)

MOF = multiple organ failure.
Two-sided Wilcoxon rank-sum with normal approximation and continuity correlation
[a]$p = 0.044$; Fisher exact test
[b]$p = 0.008$,
[c]$p < 0.001$; p values based on comparing those in group to those not in the group.

TNF—coefficient = 1.5018, SE = 0.8807, $z = 1.71$, $p = 0.088$, 95% CI = –0.2244 to 3.2281).

Overall, cases with MOF and one or more inflammation phenotypes had higher peak CRP ($p = 0.009$) and peak ferritin levels ($p < 0.001$) than cases of MOF without any phenotype, or cases with single organ failure (**Table 3**). Peak ferritin levels were higher in cases with immunoparalysis-associated MOF ($p < 0.05$), TAMOF ($p < 0.05$), or SMOF with new hepatobiliary dysfunction ($p > 0.05$) compared to cases of MOF without any of these inflammation phenotypes (Table 3). Peak CRP levels were higher in cases with immunoparalysis-associated MOF (p < 0.05) or TAMOF ($p < 0.05$), but not in cases with SMOF compared to cases with MOF without any of the phenotypes (Table 3).

MAS occurred in 11 cases (11%) overall and was more common in the MOF population with one or more of the phenotypes (10/37 cases) compared to the MOF population without the phenotypes (1/38 cases) ($p = 0.003$). Of the 11 cases with MAS, six had immunoparalysis-associated MOF, seven had TAMOF, and two had SMOF (Fig. 1, *bottom*). These 11 cases occurred in 11 different children.

There were eight deaths (8%). The presence of an inflammation phenotype was independently associated with outcome, whereas PRISM III illness severity was not (PRISM: coefficient = 0.0026, SE = 0.0033, $t = 0.80$, $p = 0.423$, 95% CI = –0.0039 to 0.0092; an inflammation phenotype: coefficient = 0.1908, SE = 0.0578, $t = 3.30$, $p = 0.001$, 95% CI = 0.0760–0.3056). When compared with PRISM III, the presence of an inflammation phenotype was also associated with increased length of stay (length of stay—PRISM: coefficient = 0.0260, SE = 0.0942, $t = –0.28$, $p = 0.783$, 95% CI = –0.2130 to 0.16105; an inflammation phenotype: coefficient = 6.8936, SE = 1.6487, $t = 4.18$, $p < 0.001$, 95% CI = 3.6214–10.1659).

TABLE 3. Peak Ferritin and *C*-Reactive Protein Levels for Single Organ Failure, Multiple Organ Failure With and Without Inflammation Phenotypes, and Each Multiple Organ Failure Inflammation Phenotype

Organ Failure Status	Statistic	Peak Ferritin (ng/mL)	Peak *C*-Reactive Protein (mg/dL)
Single organ failure ($n = 25$)	Minimum, maximum	40, 2,470	0.2, 27.9
	Median	200	3.0
	Q1–Q3	120–420	1.5–6.0
	Mean (SD)	406 (537)	5.6 (6.6)
MOF without inflammation phenotypes ($n = 38$)	Minimum, maximum	30, 6,100	0.2, 30.1
	Median	185	5.1
	Q1–Q3	100–360	1.8–11.2
	Mean (SD)	550 (1,180)	7.9 (8.0)
MOF with inflammation phenotypes ($n = 37$)	Minimum, maximum	50, 48,820	0.2, 51.8
	Median	670	10.6
	Q1–Q3	240–2,610	3.6–21.7
	Mean (SD)	3,655 (8,654)	13.6 (12.5)
	p	< 0.001	0.009
Immunoparalysis-associated MOF ($n = 24$)	Minimum, maximum	50, 48,820	0.3, 51.8
	Median	885	13.4
	Q1–Q3	285–3,205	3.0–22.6
	Mean (SD)	4,743 (10,501)	15.8 (14.1)
	p	< 0.001	0.015
Thrombocytopenia-associated MOF ($n = 15$)	Minimum, maximum	50, 48,820	0.5, 51.8
	Median	1,100	15.0
	Q1–Q3	390–4,770	4.1–26.2
	Mean (SD)	5,868 (12,510)	17.4 (15.3)
	p	0.004	0.017
Sequential MOF with new hepatobiliary dysfunction ($n = 6$)	Minimum, maximum	130, 48,820	0.2, 11.9
	Median	1,025	4.7
	Q1–Q3	150–8,930	3.6–7.3
	Mean (SD)	10,013 (19,310)	5.4 (3.9)
	p	0.223	0.597

MOF = multiple organ failure.
Q1 and Q3 represent interquartile range. *p* values are based on a Wilcoxon-Mann-Whitney test comparing those in the group to those that are not in the group.

All the deaths occurred among cases in whom MOF with one or more of the phenotypes developed, with all but one death observed in the presence of unresolving MAS. Mortality rates were higher in cases with MOF and one or more inflammation phenotypes compared to cases with MOF without any phenotype (8/37 vs 0/38; $p = 0.002$) (**Fig. 2**). In cases in whom MAS developed, mortality occurred in 64% (7/11) compared with 1% (1/89) of cases without MAS ($p < 0.001$) (Fig. 2). Among the cases of MOF with one or more inflammation phenotypes and MAS mortality was 70% (7/10), compared with 4% (1/27) of MOF cases with one or more inflammation phenotypes without MAS ($p < 0.001$) (Fig. 2). **Figures 3** and **4** show the relationships between the confirmatory biomarkers in each pathobiology phenotype in survivors and nonsurvivors over the first 4 samplings. **Supplementary Figure 1** (Supplemental Digital Content 2, http://links.lww.com/PCC/A403; **legend**, Supplemental Digital Content 1, http://links.lww.com/PCC/A402) shows three representative patients who resolved their

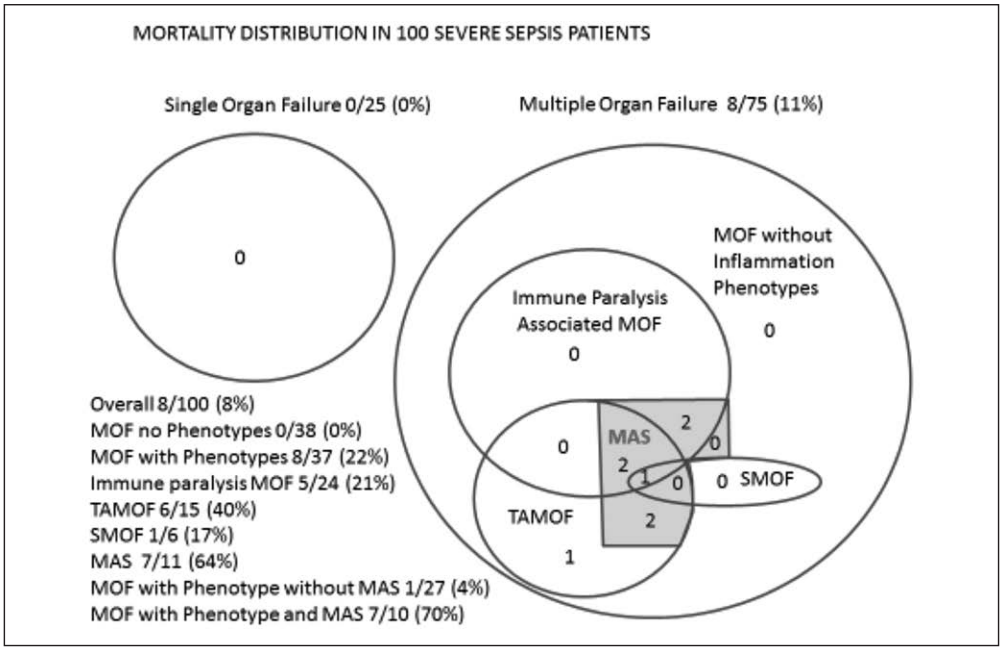

Figure 2. Mortality distribution in the severe sepsis population. All mortality occurred among multiple organ failure (MOF) cases with one or more of the inflammation phenotypes (Fisher exact test—MOF with phenotypes 8/37, 28% vs MOF without phenotypes 0/38, 0%; $p = 0.002$) and was highest among those who developed macrophage activation syndrome (MAS) (Fisher exact test—MOF with inflammation phenotypes and MAS 7/10, 70% vs MOF with inflammation phenotypes without MAS 1/27, 4%; $p < 0.001$). SMOF = sequential MOF, TAMOF = thrombocytopenia-associated MOF.

isolated inflammation pathobiology phenotype and did not succumb to MAS. **Supplementary Figure 2** (Supplemental Digital Content 3, http://links.lww.com/PCC/A404; legend, Supplemental Digital Content 1, http://links.lww.com/PCC/A402) shows two representative patients who experienced worsening of their inflammation pathobiology phenotype(s) and subsequently succumbed with MAS.

Two previously healthy patients died in the study (2/41 cases = 5% case mortality rate). Pertussis and *Streptococcus Pneumoniae* pneumonia developed in patient 1, and patient 1 died with unremitting TAMOF and MAS. *Staphylococcus aureus* and Penicillium pneumonia developed in patient 2, and patient 2 died with unremitting immunoparalysis, TAMOF, and MAS. Six chronically ill children also died (6/59 cases = 10% case mortality rate). Patient 3 had acute on chronic hemorrhagic pancreatitis and died with unremitting TAMOF without MAS. Patient 4 had pre B-cell leukemia and neutropenia with alpha-hemolytic Streptococcus and Candida septicemia and died with unremitting immunoparalysis and MAS. Patient 5 had acute myelogenous leukemia and aplastic anemia with *Enterococcus* and Stenotrophomonas pneumonia and died with unremitting immunoparalysis, TAMOF, and MAS. Patient 6 had acute lymphocytic leukemia and neutropenia with *Escherichia coli* septicemia and died with unremitting immunoparalysis and MAS. Patient 7 had an orthotopic liver transplant with adenovirus, Epstein-Barr virus, and vancomycin-resistant Enterococcus septicemia and died with unremitting immunoparalysis, SMOF, TAMOF, and MAS. Patient 8 had a small bowel transplant with graft versus host disease and methicillin-resistant *S. aureus* and VRE septicemia and died with unremitting TAMOF and MAS.

DISCUSSION

In our population sample, one or more of the inflammation pathobiology phenotypes were observed in one out of three severe sepsis cases overall, and one of two cases of sepsis-induced MOF. The children with one or more of these phenotype(s) had more systemic inflammation, and an increased proclivity to develop features of MAS and death compared to those without any of the phenotypes.

Conditions such as severe sepsis can lead to release of damage-associated molecular patterns and pathogen-associated molecular proteins that contribute to exaggerated inflammation. This induces endotheliopathy with microvascular thrombosis, epithelial cell dysfunction, mitochondrial dysfunction with mitophagy and mitochondrial dysoxia, and apoptosis of lymphocytes with depression of monocyte/macrophage function, all of which result in multiple organ dysfunction syndrome (MODS)/MOF. In addition to providing immediate organ support therapies to keep the patient alive, the clinician must also remove the source of inflammation for MOF to resolve. This includes use of proper antimicrobials and surgical source control. In theory, removal of the source of inflammation facilitates improved organ function as microvascular thrombosis resolves and endothelium regenerates, lymphocyte apoptosis stops and innate immune function recovers, epithelial cells regenerate and lung and hepatobiliary function recovers, and mitogenesis restores metabolic homeostasis.

The tenet of our present investigation is that there are pathobiology-driven subsets of patients with decreased ability to control inflammation and resolve MOF, who can be recognized by clinical phenotypes and confirmed by specific biomarkers, for whom therapies directed to respective inflammation pathobiology might facilitate control of inflammation and reversal of MOF. Before testing this unproven hypothesis in clinical trials, it is first necessary to determine whether pathobiology-driven subsets are associated with adverse outcomes in septic children.

Immunoparalysis is defined by immunodepression beyond 3 days and was observed in 24% of our severe sepsis population sample with 21% mortality. Similar to adults, it was found in association with older age, chronic illness, or cancer. Treatments including radiation, dexamethasone, chemotherapy, and other

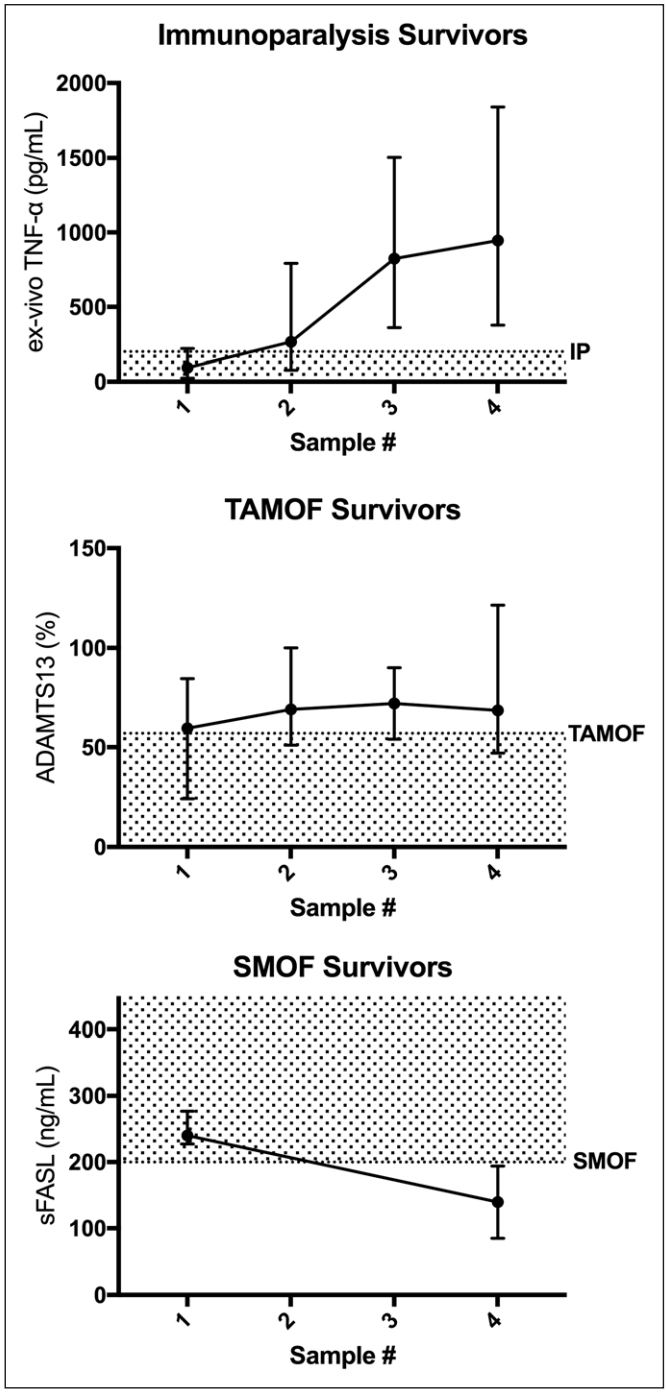

Figure 3. First four time point (days 2–12) confirmatory biomarker levels for survivors with each inflammation pathobiology phenotype (ex vivo tumor necrosis factor [TNF] response pg/mL, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity % of control, and soluble Fas ligand [sFASL] pg/mL median with 5th and 95th percentile). Granulocyte macrophage-colony stimulating factor (GM-CSF) administration and withdrawal of immunosuppressants occurred in eight of 19 immunoparalysis survivors. Plasma exchange was given to six of nine thrombocytopenia-associated MOF (TAMOF) survivors. No patients received anakinra, eculizumab, rituximab, or etoposide. SMOF = sequential MOF.

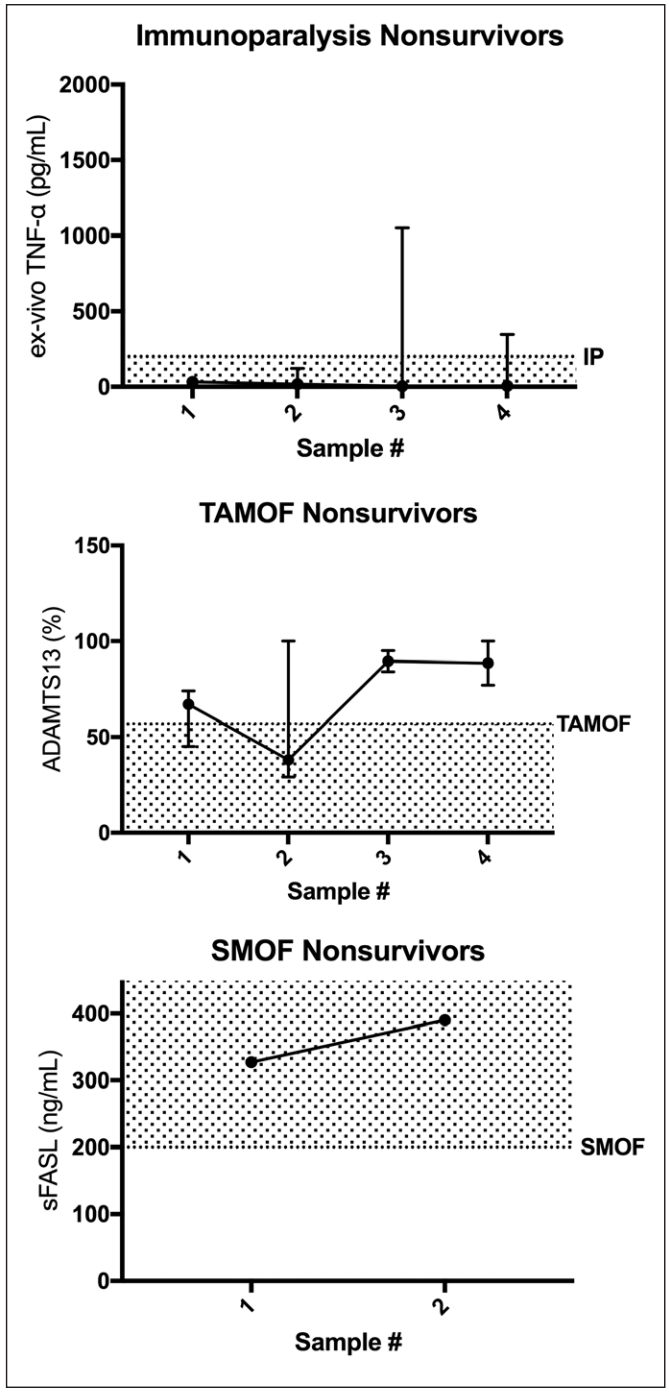

Figure 4. First four time point (days 2–12) confirmatory biomarker levels for nonsurvivors with the inflammation pathobiology phenotypes (ex vivo tumor necrosis factor [TNF] response pg/mL, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity % of control, and soluble Fas ligand [sFASL] pg/mL median with 5th and 95th percentile). Granulocyte macrophage-colony stimulating factor (GM-CSF) administration and withdrawal of immunosuppressants occurred in 0 of five immunoparalysis nonsurvivors. Plasma exchange was given to four of six thrombocytopenia-associated MOF (TAMOF) nonsurvivors. No patients received anakinra, eculizumab, rituximab, or etoposide. SMOF = sequential MOF.

immunosuppressants can induce this phenotype, leading some investigators to suggest tapering immunosuppressants when immunoparalysis occurs (19–23). In a series of small studies, GM-CSF has been found to reverse immunoparalysis, improve 7-day cure rates, and reduce secondary infections in adult patients with sepsis (4, 24–27). The epidemiologic relevance of this sepsis phenotype in children has been corroborated by a multiple center study during the *H1N1* influenza A epidemic

which demonstrated that *S. aureus* coinfection and death were associated with a low ex vivo TNF-α response to LPS stimulation with a receiver operating characteristics area under the curve equal to 0.97 (8). In addition to this functional biomarker (4), lymphopenia (3) and decreased monocyte HLA-DR expression (4) are also being used to define this phenotype in ongoing adult clinical trials (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/PCC/A402).

TAMOF occurs at any time in the PICU. It was observed in 15% of our severe sepsis cases with a mortality rate of 40%. DIC, aHUS/TMA, and TTP represent the full spectrum of this phenotype (9–13). Therapeutic plasma exchange restores ADAMTS13 levels, removes ultra large von Willebrand factor multimers, reverses TMA, and improves outcomes in adult patients with DIC, aHUS/TMA, and TTP (28, 34, 35). Multiple mutations in the inhibitory component of complement, particularly complement H, have been found to contribute to over activation of complement and coagulation in aHUS/TMA leading to use of the C5A monoclonal antibody (eculizumab) as well as plasma exchange therapy in clinical practice in adults and children (29). None of the TAMOF patients in our study received eculizumab.

The SMOF phenotype with new hepatobiliary dysfunction was observed in 6% of our severe sepsis cases making it the least common inflammation pathobiology. Cases with this phenotype had a mortality of 17%. The SMOF with new hepatobiliary dysfunction phenotype is diagnosed when respiratory distress is followed several days later by the development of liver dysfunction and is confirmed by circulating sFasL levels greater than 200 pg/mL (14). In vitro, sFasL induces liver cell apoptosis/necrosis at concentrations greater than 500 pg/mL (17, 18). This phenotype has been associated with several disorders including virus-associated hemophagocytosis and PTLD. Virus-associated hemophagocytosis can be treated with IV immunoglobulin, methylprednisolone, and antiviral therapies. PTLD can be treated with immunosuppressant tapering, antiviral medication, and administration of rituximab (CD20/B-cell antibody) to destroy the EBV reservoir, followed by IV immunoglobulin for hypogammaglobulinemia (30, 31). There was only child with PTLD in our study and she was not treated with rituximab.

Features of MAS occurred in 11% of our severe sepsis patients in strong association with the presence of one or more of the three inflammation phenotypes. The 64% mortality rate observed in our pediatric MAS subset is similar to the 65.7% mortality we reported in a placebo-treated adult severe sepsis subset with features of MAS (33). Mortality in this adult severe sepsis MAS subset was reduced to 34.6% with IL-1 receptor blockade treatment (33). The IL-1 receptor antagonist has also been reported to be effective in reversing MAS in a small nonrandomized case series of critically ill children with MOF (36). Demirkol et al (37) reported that children with five and six organ failure MAS attained 100% survival when treated with daily plasma exchange and a regimen of IV immunoglobulin and/or methylprednisolone compared to only 50% survival when treated with a more immunosuppressive regimen that included dexamethasone, etoposide, or other chemotherapy (37). None of the children with MAS in our study received anakinra or the IV immunoglobulin/methylprednisolone therapeutic strategy.

There are several limitations in our study. First, our decision to enroll on the second day of severe sepsis enriched sampling of patients in whom MOF developed because MOF rarely develops in patients in whom severe sepsis improves by day 2. Second, the phenotypes and biomarkers tracked in our study must be considered in relation to others published in the literature. Using unbiased methodology, Knox et al (38) defined four phenotypes in adult sepsis-induced MODS including one phenotype with increased creatinine and another phenotype with liver disease. It remains unknown whether these two phenotypes overlap in any way with the TAMOF and SMOF with new hepatobiliary dysfunction phenotypes, respectively. Wong et al (39) described a pediatric septic shock subclass with depressed adaptive immune and glucocorticoid signaling gene expression. It also remains unknown whether this subclass overlaps in any way with the immunoparalysis phenotype. These same investigators recently used their unbiased PERSERVERE biomarkers to identify pediatric septic shock TAMOF patients with the highest mortality risk, and then developed a new predictive model to be used for patient stratification in future plasma exchange clinical trials in the TAMOF phenotype (40). Compared with the biomarkers used by Wong et al (39), our biomarkers are very basic and generic. Third, our decision to enroll consecutive cases rather than exclude children who had previous hospitalizations for severe sepsis led to four children being analyzed in two separate hospital stays. The statistical results remained similar when eliminating the second admission, as to when including the second admission of these children. Fourth, it is important to emphasize that we do not hold that the presence of these "inflammation phenotypes" in children with sepsis means that therapies being trialed for diseases/symptoms that have a primary inflammation etiology should be applied to patients with sepsis who have a similar inflammatory response to infection without appropriate evaluation in clinical sepsis trials. We do not claim that children with sepsis and features of PTLD will benefit from rituximab, or that children with sepsis and features of aHUS will benefit from eculizumab, or that children with sepsis and features of TTP will benefit from plasma exchange, or that children with sepsis and features of MAS will benefit from anakinra. Instead, we raise the question whether future multiple center trials might be considered to address these research possibilities.

CONCLUSIONS

In summary, we present proof of concept that during severe sepsis, children with one or more of three hypothetical inflammation pathobiology phenotypes have higher peak levels of very basic and generic biomarkers of systemic inflammation, with increased proclivity to develop features of MAS and death. Because our approach to phenotype categorization remains hypothetical, the phenotypes identified need to be confirmed in multicenter studies of pediatric MODS.

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