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Three hypothetical inflammation pathobiology phenotypes and pediatric sepsis-induced multiple organ failure outcome

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Abstract

Objective—We hypothesize that three inflammation pathobiology phenotypes are associated with increased inflammation, proclivity to develop features of Macrophage Activation Syndrome (MAS), and Multiple Organ Failure (MOF) related death in pediatric severe sepsis.

Design—Prospective cohort study comparing children with severe sepsis and any of three phenotypes; 1) Immune paralysis Associated MOF (whole blood ex vivo TNF response to endotoxin < 200 pg/mL), 2) Thrombocytopenia Associated MOF (new onset thrombocytopenia with acute kidney injury and ADAMTS13 activity < 57%), and / or 3) Sequential MOF with Hepatobiliary Dysfunction (respiratory distress followed by liver dysfunction with sFASL > 200 pg/mL), to those without any of these phenotypes.

Setting—Tertiary children's hospital pediatric intensive care unit.

Patients—One hundred consecutive severe sepsis admissions.

Interventions—Clinical data was recorded daily and blood was collected twice weekly.

Measurements and Main Results—Seventy five cases developed MOF and 8 died. MOF cases with any of the three inflammation phenotype(s) (n = 37) had higher inflammation (C-reactive protein p=0.009 and ferritin p<0.001) than MOF cases without any of these phenotypes (n = 38) or cases with only single organ failure (n = 25). Development of features of MAS and death were more common among MOF cases with any of the phenotypes (MAS 10/37, 27%; death 8/37,

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22%) compared to MOF cases without any phenotype (MAS 1/38, 3%, $p=0.003$, death 0/38, 0%, $p=0.002$).

Conclusion—Our approach to phenotype categorization remains hypothetical and the phenotypes identified need to be confirmed in multicenter studies of pediatric MODS.

Keywords

Pediatric Sepsis; Immunoparalysis; Thrombocytopenia Associated Multiple Organ Failure; Sequential Multiple Organ Failure; Macrophage Activation Syndrome

Introduction

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) Immune paralysis Associated MOF,^{3–8} 2) Thrombocytopenia Associated MOF,^{9–13} and 3) Sequential MOF with new Hepatobiliary Dysfunction.^{14–18}

Children with *Immune paralysis 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 HLA-DR expression, or lymphopenia for more than three days.^{3,4} Immune function can be restored with immune modulation or immune suppressant tapering.^{4,7,19–27} Hyper-inflammation 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 Immune paralysis with immune suppressant tapering or 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 *Thrombocytopenia Associated MOF* 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} Hyper-inflammation in these children has been associated with complement over-activation in thrombotic thrombocytopenic purpura/atypical hemolytic uremic syndrome (TTP/aHUS) as well as necrosis related to microvascular thrombosis in disseminated intravascular coagulation (DIC). Eculizumab (C5a antibody) is an FDA approved drug for aHUS that reverses hyper-complementemia driven inflammation.²⁹

Children with *Sequential MOF* 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 lympho-proliferation with soluble Fas ligand (sFasL) release, hemophagocytosis, and sFasL-mediated liver injury which can respond to varied treatment strategies.^{14–19,30,31} Hyper-inflammation 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 post transplantation 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 and colleagues define MAS in children with uncontrolled systemic juvenile idiopathic arthritis by the development of a unique ‘hyper-inflammation’ MOF organ failure pattern which includes new onset hepatobiliary dysfunction, disseminated intravascular coagulation (DIC), and hyper-ferritinemia.³² 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, Appendix). 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 UPMC Institutional Review Board (IRB). 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 2009–2014. 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 ≥ 44 weeks gestation and < 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 8 samples for batch analysis). 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 ELISA (R&D Systems, Minneapolis, MN, USA). ADAMTS13 activity was measured by a commercial assay kit (Immucor GTI Diagnostics/Lifecodes Inc, Waukesha WI, USA; Immucor.com). Ferritin and C - reactive protein were measured in the CHP-UPMC clinical laboratory using the same standard operating procedure used for clinical specimens in our hospitals. The peak Ferritin and CRP sample level from the total number of samples was 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 white blood cell count (> 12,000 mm³ or < 4,000 mm³ 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 al¹⁴ (Cardiovascular - need for cardiovascular agent infusion support, Pulmonary- need for mechanical ventilation support with the ratio of the arterial partial pressure of oxygen and the fraction of inspired oxygen (PaO₂/FiO₂) < 300 without this support, Hepatic – total bilirubin > 1.0 mg/dL and alanine aminotransferase (ALT) > 100 units/L, Renal – serum creatinine > 1.0 mg/dL and oliguria (urine output < 0.5 mL/kg/hr), Hematologic - thrombocytopenia < 100,000/mm³ and prothrombin time INR > 1.5 × normal, and Central Nervous System – Glasgow Coma Scale (GCS) < 12 in absence of sedatives). *Severe Sepsis* was defined by the presence of sepsis and one or more organ failures. *Multiple Organ Failure* was defined by the development of two or more organ failures. *Immune paralysis Associated MOF*,⁴ *Thrombocytopenia Associated MOF*,⁹ *Sequential MOF with new Hepatobiliary Dysfunction*,¹⁴ and *Macrophage Activation Syndrome*,^{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's 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's exact test was used to compare the development of MAS and mortality between groups. A p value < 0.05 was considered statistically significant. A secondary analysis was performed excluding the four patients who were admitted twice in the study.

Results

Ninety-six patients comprised 100 consecutive PICU severe sepsis cases. The average age of the children was 5.8 years (standard deviation (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, 9 fungal) and 25 were culture negative. The average 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, 25 had single organ failure, 38 developed MOF without any of the three inflammation phenotypes (mean OFI = 2.3, SD = 0.5) and 37 developed MOF 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 24/37 Immune paralysis associated MOF, 15/37 Thrombocytopenia associated MOF and 6/37 Sequential MOF (Figure 1, top panel). Thirty of these 37 had one distinct phenotype, 6/37 had two phenotypes overlapping, and 1/37 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 was found in the Immune paralysis associated MOF phenotype (Table 2). Immune paralysis associated MOF was more likely to be observed in cases with increased age (7.8 years $\pm$ 5.97 vs 5.2 years $\pm$ 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 to Absolute Lymphocyte Count (ALC) $< 1,000/\mu\text{L}$, a low *ex vivo* TNF production was associated with longer length of stay (Length of Stay - ALC $< 1,000$: Coefficient = -1.5211, Standard Error = 1.8112, $t = -0.84$, p value = 0.403, 95% Confidence Interval = -5.1160 - 2.07367; Low *ex vivo* TNF - Coefficient = 5.9973, Standard Error = 1.9814, $t = 3.03$, p value = 0.03, 95% Confidence Interval = 2.0646 - 9.9301) and a non-significant tendency towards increased mortality (Mortality - ALC $< 1,000$: Coefficient = .5888, Standard Error = .8841, $z = 0.67$, p value = 0.505, 95% Confidence interval = -1.1441 - 2.3218; Low *ex vivo* TNF - Coefficient = 1.5018, Standard Error = .8807, $z = 1.71$, p value = 0.088, 95% Confidence Interval = -.2244 - 3.2281).

Overall, cases with MOF and one or more inflammation phenotype 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 Immune paralysis Associated MOF ($p < 0.05$), Thrombocytopenia Associated MOF ($p < 0.05$), or Sequential MOF 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 Immune paralysis Associated MOF ($p < 0.05$) or Thrombocytopenia Associated MOF ($p < 0.05$), but not in cases with Sequential MOF compared to cases with MOF without any of the phenotypes (Table 3).

Macrophage Activation Syndrome 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, 6 had Immune paralysis Associated MOF, 7 had Thrombocytopenia Associated MOF, and 2 had Sequential MOF (Figure 1, Bottom Panel). These 11 cases occurred in eleven 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 = .0026, Standard Error = .0033, $t = 0.80$, P value 0.423, 95% confidence interval = $-.0039 - .0092$; an Inflammation Phenotype: coefficient .1908, Standard Error = .0578, $t = 3.30$, p value = 0.001, 95% confidence interval = $.0760 - .3056$). As compared to PRISM III the presence of an Inflammation Phenotype was also associated with increased length of stay (Length of Stay - PRISM: Coefficient = .0260, Standard Error = .0942, $t = -0.28$, p value = 0.783, 95% Confidence Interval = $-.2130 - .16105$; an Inflammation Phenotype: Coefficient = 6.8936, Standard Error = 1.6487, $t = 4.18$, $p < 0.001$, 95% Confidence Interval = $3.6214 - 10.1659$).

All the deaths occurred among cases who developed MOF with one or more of the phenotypes, 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 phenotype compared to cases with MOF without any phenotype (8/37 vs 0/38; Figure 2, $p = 0.002$). In cases that developed MAS, mortality occurred in 64% (7/11) compared to 1% (1/89) of cases without MAS (Figure 2; $p < 0.001$). Among the cases of MOF with one or more inflammation phenotype and MAS mortality was 70% (7/10), compared to 4% (1/27) of MOF cases with one or more inflammation phenotype without MAS (Figure 2; $p < 0.001$). Figures 3 and 4 show the relationships between the confirmatory biomarkers in each pathobiology phenotype in survivors and non-survivors over the first 4 samplings. Figure 5 (supplementary) shows three representative patients who resolved their isolated inflammation pathobiology phenotype and did not succumb to MAS. Figure 6 (supplementary) 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). Patient 1 developed Pertussis and Streptococcus Pneumoniae pneumonia and died with unremitting TAMOF and MAS. Patient 2 developed Staphylococcus Aureus and Penicillium pneumonia and died with unremitting Immune paralysis, 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 Immune paralysis and MAS. Patient 5 had AML and aplastic anemia with Enterococcus and Stenotrophomonas pneumonia and died with unremitting Immune paralysis, TAMOF, and MAS. Patient 6 had ALL and neutropenia with Escherichia coli septicemia and died with unremitting Immune paralysis and MAS. Patient 7 had an orthotopic liver transplant with Adenovirus, EBV, and VRE septicemia and died with unremitting Immune paralysis, SMOF, TAMOF, and MAS. Patient 8 had a small

bowel transplant with graft versus host disease and MRSA 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 DAMPS (damage associated molecular patterns) and PAMPS (pathogen associated molecular patterns) 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/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.

Immune paralysis, is defined by immune depression beyond three 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 immune suppressants can induce this phenotype, leading some investigators to suggest tapering immune suppressants when Immune paralysis occurs.^{19–23} In a series of small studies, GM-CSF has been found to reverse Immune paralysis, improve seven 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 *Staphylococcus aureus* co-infection and death was 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

lymphopenia³ and decreased monocyte HLA-DR expression⁴ are also being used to define this phenotype in ongoing adult clinical trials (Supplementary Table, Appendix).

Thrombocytopenia Associated MOF occurs at any time in the PICU. It was observed in 15% of our severe sepsis cases with a mortality rate of 40%. Disseminated Intravascular Coagulation, aHUS / TMA, and TTP represent the full spectrum of this phenotype.^{9–13} Therapeutic plasma exchange restores ADAMTS13 levels, removes ultra large vWF multimers, reverses thrombotic microangiopathy, 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 Sequential MOF 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 Sequential MOF 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 > 200 pg/mL.¹⁴ *In vitro*, sFasL induces liver cell apoptosis / necrosis at concentrations > 500 pg/mL.^{17,18} This phenotype has been associated with several disorders including virus associated hemophagocytosis and post-transplant lymphoproliferative disease. Virus associated hemophagocytosis can be treated with intravenous immunoglobulin (IVIG), methylprednisolone, and anti-viral therapies. Post-transplant lymphoproliferative disease can be treated with immune suppressant tapering, anti-viral medication and administration of rituximab (CD20 / B-cell antibody) to destroy the EBV reservoir, followed by IVIG for hypogammaglobulinemia.^{30,31} There was only child with PTLN in our study and she was not treated with Rituximab.

Features of Macrophage Activation Syndrome 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 non-randomized case series of critically ill children with MOF.³⁶ Demirkol and colleagues reported that children with 5 and 6 organ failure MAS attained 100% survival when treated with daily plasma exchange and a regimen of IVIG and / or methylprednisolone compared to only 50% survival when treated with a more immune suppressive regimen that included dexamethasone, etoposide, or other chemotherapy.³⁷ None of the children with MAS in our study received Anakinra or the IVIG / Methylprednisone 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 who developed MOF because patients who improve severe sepsis by day two rarely develop MOF. Second, the phenotypes and

biomarkers tracked in our study must be considered in relation to others published in the literature. Using unbiased methodology Knox and colleagues defined four phenotypes in adult sepsis induced MODS³⁸ 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 and colleagues described a pediatric septic shock subclass with depressed adaptive immune and glucocorticoid signaling gene expression.³⁹ It also remains unknown whether this subclass overlaps in any way with the Immune paralysis 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 to the biomarkers used by Wong and colleagues, 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 PTLT 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.

Conclusion

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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SINGLE and MULTIPLE ORGAN FAILURE DISTRIBUTION IN 100 SEVERE SEPSIS PATIENTS

MAS DISTRIBUTION IN 100 SEVERE SEPSIS PATIENTS

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 MOF patients had one or more of the three inflammation phenotypes (Top panel). 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's exact test $p = 0.003$ (Bottom Panel). Single organ failure (SOF); Multiple organ failure (MOF); Immune paralysis Associated MOF (IP MOF); Thrombocytopenia Associated MOF (TAMOF); Sequential MOF (SMOF); Macrophage Activation Syndrome (MAS)

MORTALITY DISTRIBUTION IN 100 SEVERE SEPSIS PATIENTS

Figure 2. Mortality distribution in the severe sepsis population

All mortality occurred among MOF cases with one or more of the inflammation phenotypes (Fisher's 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 MAS (Fisher's exact test - MOF with inflammation phenotypes and MAS 7/10, 70% vs MOF with inflammation phenotypes without MAS 1/27, 4%; $p < 0.001$). SOF= Single Organ Failure; MOF = Multiple Organ Failure TAMOF = Thrombocytopenia Associated MOF; SMOF = Sequential MOF; IP MOF = Immune paralysis Associated MOF; MAS = Macrophage Activation Syndrome

Figure 3. First four time point (Days 2 through 12) confirmatory biomarker levels for survivors with each inflammation pathobiology phenotype (ex vivo TNF response pg/mL, ADAMTS13 activity % of control, and sFASL pg/mL median with 5th and 95th percentile)

GM-CSF administration and withdrawal of immune suppressants occurred in 8 of 19 Immune Paralysis survivors. Plasma exchange was given to 6 of 9 TAMOF survivors. No patients received Anakinra, Eculizumab, Rituximab, or Etoposide.

Figure 4. First four time point (Days 2 through 12) confirmatory biomarker levels for non-survivors with the inflammation pathobiology phenotypes (ex vivo TNF response pg/mL, ADAMTS13 activity % of control, and sFASL pg/mL median with 5th and 95th percentile) GM-CSF administration and withdrawal of immune suppressants occurred in 0 of 5 Immune Paralysis non-survivors. Plasma exchange was given to 4 of 6 TAMOF non-survivors. No patients received Anakinra, Eculizumab, Rituximab, or Etoposide.

Table 1

Clinical and biomarker criteria used to define inflammation phenotypes and macrophage activation syndrome

Group	Clinical criteria	Confirmatory Biomarker
Immune paralysis Associated MOF (Immune depression)	1) Beyond third day of critical illness with MOF	Whole blood <i>ex vivo</i> TNF response to LPS < 200 pg/mL
Thrombocytopenia Associated MOF (Thrombotic Micro-angiopathy)	1) New thrombocytopenia < 100 K, or if baseline platelet count < 100 K then 50% decrease from baseline 2) Elevated LDH > 250 u/L 3) Creatinine > 1 mg/dL and oliguria	ADAMTS 13 activity < 57% of control
Sequential MOF with new Hepatobiliary Dysfunction (Virus / lympho-proliferative disease disease)	1) PaO ₂ /FiO ₂ < 300 with need for mechanical ventilation 2) Followed days later by new onset hepatic dysfunction ALT > 100u/L + Bilirubin > 1 mg/dL	sFasL > 200 pg/mL
Macrophage Activation Syndrome (Hyperinflammation common end pathway)	1) Disseminated Intravascular Coagulation with Platelet count < 100 K and INR > 1.5 2) New hepatic dysfunction ALT > 100 u/L + Bilirubin > 1 mg/dL	Ferritin > 500 ng/mL

MOF = multiple organ failure; LPS = lipopolysaccharide; pg = picogram; mg = milligram; L = liter; mL = milliliter; dL = deciliter; u = unit; K = 1,000; ADAMTS 13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; LDH = lactate dehydrogenase; PaO₂ = partial pressure of oxygen; FiO₂ = fractional inspired oxygen; ALT = Alanine Amino transferase; sFasL = soluble FAS ligand; ng = nanogram; INR = international normalized ratio

Table 2

Baseline characteristics of each inflammation phenotype

Baseline Status	IPMOF		TAMOF		SMOF/ Hepatobiliary Dysfunction		
	No	Yes	No	Yes	No	Yes	
	N = 76	N=24	N = 85	N = 15	N = 94	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 Ill							
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%)	

Baseline Status	IPMOF		TAMOF		SMOF/ Hepatobiliary Dysfunction	
	No	Yes	No	Yes	No	Yes
Yes	N = 76 17 (22%)	N=24 8 (33%)	N = 85 21 (25%)	N = 15 4 (27%)	N = 94 24 (26%)	N = 96 1 (17%)

Two-sided Wilcoxon rank-sum with normal approximation and continuity correlation

- ^a p-value = 0.044;
Fishers exact test
^b p-value = 0.008,
^c p-value < 0.001; p values based on comparing those in group to those not in the group.

IP - Immune Paralysis; MOF – multiple organ failure; TAMOF – Thrombocytopenia associated MOF; SMOF – Sequential MOF; Chronic III – chronic illness at admission.

Table 3

Peak ferritin and CRP levels for single organ failure, multiple organ failure with and without inflammation phenotypes, and each MOF inflammation phenotype.

Organ Failure Status	Statistic	Peak Ferritin ng/mL	Peak CRP mg/dL
Single Organ Failure (n = 25)	Min, Max	40, 2470	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)	Min, Max	30, 6100	0.2, 30.1
	Median	185	5.1
	Q1, Q3	100, 360	1.8, 11.2
	Mean (SD)	550 (1180)	7.9 (8.0)
MOF with Inflammation Phenotypes (n = 37)	Min, Max	50, 48820	0.2, 51.8
	Median	670	10.6
	Q1, Q3	240, 2610	3.6, 21.7
	Mean (SD)	3655 (8654)	13.6 (12.5)
	p-value	< 0.001	0.009
IP MOF (n = 24)	Min, Max	50, 48820	0.3, 51.8
	Median	885	13.4
	Q1, Q3	285, 3205	3.0, 22.6
	Mean (SD)	4743 (10501)	15.8 (14.1)
	p-value	< 0.001	0.015
TAMOF (n = 15)	Min, Max	50, 48820	0.5, 51.8
	Median	1100	15.0
	Q1, Q3	390, 4770	4.1, 26.2
	Mean (SD)	5868 (12510)	17.4 (15.3)
	p-value	0.004	0.017
SMOF with new Hepatobiliary Dysfunction (n = 6)	Min, Max	130, 48,820	0.2, 11.9
	Median	1025	4.7
	Q1, Q3	150, 8930	3.6, 7.3
	Mean (SD)	10013 (19310)	5.4 (3.9)
	p-value	0.223	0.597

ng = nanogram; mg = milligram; mL = milliliter; dL = deciliter; CRP = C-reactive protein; MOF = multiple organ failure; Min = minimum value; Max = maximum value; Q1, Q3 = interquartile range; SD = standard deviation; IP MOF = Immunoparalysis associated MOF; TAMOF = Thrombocytopenia Associated MOF; SMOF = Sequential MOF.

P-values are based on a Wilcoxon-Mann-Whitney test comparing those in the group to those that are not in the group