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A Systemic Inflammation Mortality Risk Assessment Contingency Table for Severe Sepsis

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Abstract

Objective—We tested the hypothesis that a c-reactive protein (CRP) and ferritin based systemic inflammation contingency table can track mortality risk in pediatric severe sepsis.

Design—Prospective cohort study

Setting—Tertiary Pediatric Intensive Care Unit

Patients—Children with 100 separate admission episodes of severe sepsis were enrolled.

Interventions—Blood samples were attained on day two of sepsis and bi-weekly for biomarker batch analysis. A 2 × 2 contingency table using CRP and ferritin thresholds was developed.

Measurements and Main Results—A CRP of 4.08 mg/dL and a ferritin of 1,980 ng/mL were found to be optimal cutoffs for outcome prediction at first sampling (n = 100) using the Youden Index. PICU mortality was increased in the 'High risk' CRP 4.08 mg/dL and Ferritin 1,980

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ng/mL category (6/13, 46.15%) compared to the 'Intermediate risk' CRP ≥ 4.08 mg/dL and Ferritin $< 1,980$ ng/mL or CRP < 4.08 mg/dL and Ferritin $\geq 1,980$ ng/mL categories (2/43, 4.65%), and the 'Low risk' CRP < 4.08 mg/dL and Ferritin $< 1,980$ ng/mL category (0/44, 0%) (OR 36.43 [95% CI: 6.16–215.21]). The 'High risk' category was also associated with the development of Immunoparalysis (OR 4.47 [95% CI 1.34–14.96]) and Macrophage Activation Syndrome (OR 24.20 [95% CI 5.50–106.54]). Sixty three children underwent sequential blood sampling; those who were initially in the 'Low risk' category ($n = 24$) and those who subsequently migrated to ($n = 19$) to the 'Low risk' category all survived, whereas those who remained in the 'At risk' categories had increased mortality ($7/20 = 35\%$; $p < 0.05$).

Conclusions—A CRP and ferritin based contingency table effectively assessed mortality risk. Reduction in systemic inflammation below a combined threshold CRP of 4.08 mg/dL and ferritin of 1,980 ng / mL appeared to be a desired response in children with severe sepsis.

Introduction

Severe sepsis is a syndrome caused by infection-induced systemic inflammation. It is the leading cause of death in children under five years old globally, and behind only accidents and trauma in the United States.^{1,2} Standard of care practice in pediatric sepsis is directed to removal of the source of infection and support of organ functions without regard to systemic inflammation. Two readily available and inexpensive systemic inflammation biomarkers, c-reactive protein (CRP)^{3–8} and ferritin,^{9–13} are known to increase with infection and sepsis, but they are not routinely monitored in standard practice in either the resource rich or poor settings.

C-reactive protein was the first pattern recognition receptor identified in the scientific literature. It binds to phosphocholine on bacteria and also to host necrotic and apoptotic cells. It then binds to macrophages and activates complement through the C1q complex facilitating enhanced clearance of microbes, and host necrotic and apoptotic cells.³ C-reactive protein levels require 12–24 hours to rise in response to infection. A rising CRP level at 72 hours is associated with a poor antibiotic response in septic adults⁴ and it is thought to reflect inadequate source control.^{5,6} In contrast, a normal CRP level for longer than 72 hours can be used as an indication to stop antibiotics in newborn 'rule out' sepsis patients.⁷

Ferritin is also released in response to sepsis and inflammation. Ferritin sequesters iron from microbes thereby inhibiting their growth. It also prevents iron mediated oxidative stress and Fenton reaction induced injury in the host. In Brazilian children with severe sepsis, a ferritin level < 200 ng/mL was associated with a 23% mortality, whereas those children with ferritin levels between 200–500 ng/mL had only a 9% mortality, supporting a protective role for ferritin.⁹ However, when ferritin levels were > 500 ng/mL, a 58% mortality was observed. These very high levels of ferritin are thought to represent macrophage activation syndrome.¹² In a study of all hospitalized children at Seattle Children's Hospital, ferritin levels $> 1,000$ ng/mL and $> 3,000$ ng/mL were associated with a stepwise increase in the risk of subsequent admission to the pediatric intensive care unit or death over the next five years regardless of cancer, rheumatologic disease, or hemolytic anemia status.¹⁰

Because CRP and ferritin reflect systemic inflammation from two different immunologic sources, namely complement activation and bacterial infection (CRP), and macrophage activation (ferritin), the purpose of our present study is to test whether there could be a possible role for using a CRP and ferritin ‘contingency table’ to assess overall systemic inflammation mortality risk in children with severe sepsis.

Patients and Methods

After attaining Institutional Review Board approval, consent was obtained from the parents of 100 consecutive pediatric cases who had severe sepsis and met inclusion criteria at the University of Pittsburgh-Children’s Hospital of Pittsburgh-UPMC site of the *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (NICHD-CPCCRN). Inclusion criteria were the presence of severe sepsis (sepsis with at least one organ failure), presence of an indwelling arterial or central venous catheter, age ≥ 44 weeks gestation and < 18 years, and desire for aggressive care. Because CRP and ferritin levels require over 24 hours to reach full response, children were enrolled and blood sampled on the second day of sepsis and then twice weekly (Monday and Thursday, or Tuesday and Friday) until the child no longer had indwelling arterial or central venous catheters for blood sampling in the pediatric intensive care unit (PICU), or 28 days had transpired in the PICU.

Sepsis was defined by the presence of two or more of the following four criteria: 1) tachycardia (Heart rate $> 90^{\text{th}}$ percentile for age), 2) tachypnea (Respiratory rate $> 90^{\text{th}}$ percentile for age), 3) abnormal temperature ($< 36^{\circ}\text{C}$ or $> 38.5^{\circ}\text{C}$), abnormal white blood cell count ($> 12,000\text{ mm}^3$ or $< 4,000\text{ mm}^3$ or $> 10\%$ immature neutrophils), plus the suspicion of infection.¹⁴ *Organ failure* was defined using the Organ Failure Index criteria of Doughty et al¹⁵ which assigns a single integer for each organ failure (Cardiovascular - need for cardiovascular support; Pulmonary-need for mechanical ventilation support with a $\text{paO}_2/\text{FiO}_2$ ratio < 300 without this support; Hepatic - bilirubin $> 1.0\text{ mg/dL}$ and alanine aminotransferase $> 100\text{ unit/L}$; Renal - creatinine $> 1.0\text{ mg/dL}$ and oliguria ($< 0.05\text{ mL/kg/hr}$); Hematologic - thrombocytopenia $< 100,000/\mu\text{L}$ and prothrombin time $> 1.5 \times$ normal; and Central Nervous System – Glasgow Coma Scale < 12 without sedation). *Severe Sepsis* was defined by the presence of sepsis and one or more of these organ failures.

Immunoparalysis associated multiorgan failure (MOF) was defined by an *ex vivo* whole blood lipopolysaccharide (LPS) stimulated tumor necrosis factor alpha (TNF α) response $< 200\text{ pg/mL}$ after 3 days in a patient with two or more organ failures.¹⁶ *Macrophage Activation Syndrome (MAS)* was defined by the presence of hepatic and hematologic failure and a ferritin level $> 500\text{ ng/mL}$ (a modification of Ravelli Criteria for MAS).¹⁷ Mortality was defined as death within the current PICU admission.

To diagnose immunoparalysis, LPS-induced TNF α production capacity was measured as previously described.¹⁶ The remaining whole blood was also spun down and the plasma was separated into aliquots and frozen for later batch analysis. The TNF α assays were performed using an enzyme-linked immunosorbent assay (ELISA) (R&D). The CRP and ferritin assays were performed using the good clinical laboratory practice facility at the Children’s Hospital

of Pittsburgh - University of Pittsburgh Medical Center. Because all analyses were performed on batched frozen samples, clinicians were blinded to all results.

The systemic inflammation mortality risk assessment contingency table was developed *a priori* as a 2×2 table with CRP $\geq$ cutoff value or $<$ cutoff value, versus Ferritin $\geq$ cutoff value or $<$ cutoff value. *A priori*, the CRP $\geq$ cutoff value and Ferritin $\geq$ cutoff value box (Box B) was considered the 'High risk' box, the CRP $<$ cutoff value and Ferritin $\geq$ cutoff value (Box A) and the CRP $\geq$ cutoff value and Ferritin $<$ cutoff value (Box D) were considered the 'Intermediate risk' boxes, and the CRP $<$ cutoff value and Ferritin $<$ cutoff value box (Box C) was considered the 'Low risk' box (Figure 1). *A priori*, migration during the PICU stay to Box C (the 'Low risk' box) was considered a desired response; whereas, migration to Box A, B, or D (the Intermediate and High risk boxes, or 'At risk' boxes) was considered an undesired response.

The overall hypothesis that mortality risk would be associated with the systemic inflammation risk biomarker profiles in the contingency table was tested. Specifically, the hypothesis that mortality in patients in the 'High risk' box would be increased compared to 'At risk' patients in the combined 'Intermediate risk' and 'Low risk' boxes at the time of first blood sampling was tested. To confirm this association, the hypothesis that the desired response, migration from the 'High risk' and 'Intermediate risk' boxes to the 'Low risk' box, would be associated with lower mortality than the undesired response, migration to or staying in the 'Intermediate risk' or 'High risk' boxes was also assessed. To examine biological plausibility, the association between the 'High risk' box and development of Immunoparalysis and Macrophage Activation Syndrome was assessed because these two conditions are thought to contribute to severe sepsis mortality.

The prediction of outcome from the continuous variables CRP and ferritin when controlling for severity of illness (PRISM) was analyzed using Logistic regression analysis with maximum likelihood estimates and Wald Chi-Square and odds ratio estimates. The Youden Index was used to establish optimal cut-off values for CRP and ferritin levels as predictors of PICU mortality for the purpose of creating the contingency table. We considered the approach of using cut points, rather than attempting to model a continuous relationship, to be appropriate given the small number of outcomes.

Fisher's exact test was used to test these hypotheses with $p < 0.05$ considered significant. Odds ratios and 95% confidence intervals (CI) were generated to evaluate the relationships between risk boxes and various outcomes. Exact methods for obtaining the confidence limits were used when observed counts were small. Logistic regression analysis was used to assess independent associations with a p value < 0.05 considered significant. Receiver Operating Characteristic (ROC) curves were generated to test the association between mortality and percent change over time in CRP or ferritin levels at the time of later blood sampling for patients who were in the 'Intermediate risk' or 'High risk' boxes at first sampling. Statistical analyses were performed using SAS software v9.3 and ROC graphs were generated using the 'ROCR' package in R version 2.15.3.

Results

One hundred consecutive severe sepsis cases were enrolled with informed parental consent in the IRB approved protocol. Thirty seven patients were in the PICU for only one sampling whereas 63 patients were in the PICU for repeated twice weekly sampling. For those with repeated sampling, the number of samples secured ranged from 2 – 8 samples attained over 6 – 28 days. The ROC curve ($n = 100$) for the continuous variable CRP ‘at first sampling’ predicting death revealed an area under the curve (AUC) of 0.770 [95% CI 0.607–0.934] (Figure 1), the ROC curve ($n = 100$) for the continuous variable ferritin ‘at first sampling’ predicting death found an AUC of 0.878 [0.751–1.000] (Figure 1), and the ROC curve ($n = 100$) for PRISM predicting death revealed an AUC of 0.669 [95% CI 0.394–0.945] (Figure 1). Logistic regression for ‘first’ CRP ($n = 100$) predicting mortality controlling for PRISM found that CRP tended to predict mortality, but PRISM did not (CRP Estimate 0.0772, Standard Error 0.0432, Wald Chi Square 3.1875, p -value 0.0742; PRISM Estimate 0.0620, Standard Error 0.0412, Wald Chi square 2.1904, p -value 0.1389) with an effect odds ratio point estimate of 1.080 for CRP [95% CI 0.992–1.176]. Logistic regression for first ferritin ($n = 100$) predicting mortality controlling for PRISM showed that ferritin predicted mortality, but PRISM did not (Ferritin Estimate 0.000530, Standard Error 0.000238, Wald Chi Square 4.9709, p -value 0.0258; PRISM Estimate 0.0273, Standard Error 0.0473, Wald Chi square 0.3337, p -value 0.5635) with an effect odds ratio point estimate of 1.001 for ferritin [95% CI 1.000–1.001]. Analysis of the ‘first sampling’ ($n = 100$) continuous variable CRP using the Youden Index demonstrated an optimal cut-off point of 4.08 mg/dL as the best predictor of mortality with a sensitivity of 1.00 and a specificity of 0.49. The analysis of ‘first sampling’ ($n = 100$) continuous variable ferritin using the Youden method established an optimal cut-off point of 1,980 ng/mL as the best predictor of mortality with a sensitivity of 0.75 and a specificity of 0.92. The contingency table was therefore built using the CRP cutoff value ≥ 4.08 mg/dL and the Ferritin cutoff value $\geq 1,980$ ng/mL.

Table 1 shows clinical characteristics according to first sampling Low risk (CRP < 4.08 mg/dL and Ferritin $< 1,980$ ng/mL), Intermediate risk (CRP ≥ 4.08 and Ferritin $< 1,980$ ng/mL or CRP < 4.08 and Ferritin $\geq 1,980$ ng/mL) and High risk (CRP ≥ 4.08 and Ferritin $\geq 1,980$ ng/mL) categories. In univariable analysis bacterial infection ($p = 0.006$; Fisher’s exact test), age in years ($p = 0.021$; Kruskal Wallis), cancer diagnosis ($p = 0.037$; Fisher’s exact test), Pediatric Risk of Mortality score (PRISM) ($p = 0.001$; Kruskal Wallis), maximum organ failure index (OFI) ($p < 0.001$ Kruskal Wallis), and mortality ($p < 0.001$; Fisher’s exact test) differed across the three risk categories. Patients with higher systemic inflammation more commonly had bacterial infection, older age, cancer, a greater severity of illness, multiple organ failure, and mortality. To explore whether the ‘Systemic Inflammation Mortality Risk’ contingency table had any independent utility in predicting death after considering these other variables, a logistic regression was performed with death as the dependent variable. In multivariable analysis, only the ‘Systemic Inflammation Mortality Risk’ category independently predicted mortality (odds ratio = 9.58; 95% confidence interval of [1.46 – 62.88], $p = 0.019$; Z statistic = 2.35); whereas, bacterial infection ($p = 0.07$; Z statistic = 1.83), age in years ($p = 0.96$; Z statistic = 0.05), cancer diagnosis ($p = 0.45$; Z statistic = 0.76), PRISM score ($p = 0.88$ Z; statistic = 0.88), and maximum OFI ($p =$

0.13; Z statistic = 1.52) did not. The Systemic Inflammation Mortality Risk contingency table predicted death even after controlling for bacterial infection, age, cancer, severity of illness, and maximum number of organ failures.

Figure 2 shows at first sampling (n = 100) that 6/13 (46.15%) with CRP ≥ 4.08 mg/dL and ferritin ≥ 1,980 ng/mL subsequently died; 0/0 (0%) with CRP < 4.08 mg/dL and ferritin ≥ 1,980 ng/mL and 2/43 (4.65%) with CRP ≥ 4.08 mg/dL and ferritin < 1,980 ng/mL died; and 0/46 (0%) with CRP < 4.08 mg/dL and ferritin < 1,980 ng/mL died (p < 0.05). Patients in the 'High risk' category had an increased risk of mortality (6/13 vs 2/87; OR 36.43 [95% CI 6.16–215.21]) as well as developing Immunoparalysis (7/13 vs 18/87, OR 4.47 [95% CI 1.34–14.96]) or MAS (7/13 vs 4/87, OR 24.20 [95% CI 5.50–106.54]). Patients in the 'Low risk' category were less likely to die (0/44; 0% vs 8/54; 14.29%, p < 0.05) and less likely to develop MAS (0/44; 0% vs 11/56; 19.64%, p < 0.05), but not statistically less likely to develop Immunoparalysis (9/44; 20.45% vs 16/56; 28.57%, p > 0.05).

Analysis of the 63 children who underwent serial sampling in the mortality risk contingency table found that 24 of the 63 children were in the 'Low risk' category at first sampling and all of these children survived. Among the 39 children who were in the 'At risk' categories at first sampling, mortality among those who subsequently migrated to the 'Low risk' category (0/19; 0%) was lower than the mortality of the children who migrated to or stayed in the 'Intermediate risk' and 'High risk' categories at last sampling (7/20; 35%) (Figure 2; p < 0.05). For illustrative purposes, Figures 4, 5, and 6 [supplement] show three representative patients who did (n = 2) and did not (n = 1) transition from the 'At risk' systemic inflammation categories to the 'Low risk' systemic inflammation category over time. Table 2 depicts the inflammation characteristics over time of the seven patients who did not survive. None of the seven non-survivors cleared their source of infection / inflammation, and all succumbed without returning to the 'Low risk' category box. The median number of days until last sampling for these non-survivors was 19 days and the median number of days until last sampling for the survivors was 9 days.

In order to assess whether positive percent change in CRP or ferritin levels over time was associated with death, and negative percent change over time was associated with survival, ROC curves were generated among the children who were in the PICU for more than one sampling who had a 'first sampling' CRP ≥ 4.08 mg/dL (n = 38) and / or a 'first sampling' ferritin ≥ 1,980 ng/mL (n = 9). The CRP ≥ 4.08 mg/dL at 'first sampling' group ROC (n = 38) for percent change in CRP at 'second sampling' time as a predictor of mortality revealed an AUC of 0.714 [95% CI 0.521–0.916]. Increase in CRP over time in 'At risk' patients was associated with death, and decrease in CRP over time was associated with survival. The ferritin ≥ 1,980 ng/mL at 'first sampling' group ROC curve (n = 9) for percent change in ferritin at second sampling time predicting mortality showed an AUC of 0.800 [95% CI 0.470–1.000]. The AUC analyses could be interpreted to suggest that increase in ferritin over time in 'At risk' patients was associated with death and decrease in ferritin level over time in 'At risk' patients was associated with survival; however, the wide confidence intervals suggest that the sample size is too small to make this conclusion. Therefore, return to the 'Low risk' category (< 1,980 ng/mL) was a more reliable predictor of outcome than percent change in ferritin in our population sample.

Discussion

In this study, a 2×2 contingency table constructed with CRP and ferritin threshold levels derived using the Youden Index for identifying best cut-offs in a ROC analysis performed well as an indicator of mortality risk in a sample of children with severe sepsis. In this contingency table, risk was classified *a priori* based on CRP ≥ 4.08 mg/dL and ferritin $\geq 1,980$ ng/mL as high risk, intermediate risk and low risk if both, one or neither CRP and ferritin were greater than threshold. As hypothesized, at first sampling, the 'High risk' category patients went on to have the highest mortality and the 'Low risk' category patients went on to have the lowest mortality. The changes in CRP and ferritin based risk categories over time tracked outcome. A transition from the 'High risk' and 'Intermediate risk' categories to the 'Low risk' category was associated with survival; whereas lack of this transition was associated with increased mortality.

Among the 'At risk' systemic inflammation categories, we found no patient in the CRP < 4.08 mg/dL and a ferritin $\geq 1,980$ ng/mL category at first sampling. A low CRP with a high ferritin can be indicative of virus infection associated hemophagocytosis, but our sample appears to be too small to fully evaluate this possibility.

The next least common group was the 'High risk' category, a CRP ≥ 4.08 mg/dL and ferritin $\geq 1,980$ ng/mL, which was associated with the highest mortality. This category accounted for only 14% of the patients, but 75% of the deaths. Biological plausibility for this relationship is supported by the observation that this category was associated with development of Immunoparalysis associated MOF and MAS. Immunoparalysis is a sepsis inflammation phenotype in which acquired immune depression leaves the host with increased inflammation in part due to a reduced ability to clear infection. This syndrome can respond to immune suppression withdrawal as well as to immune modulation with granulocyte-macrophage colony-stimulating factor (GM-CSF).^{18–28} Among our eight non-survivors, five had immunoparalysis likely due to lack of tapering of chemotherapy and immune suppressants; however, one of the previously healthy non-survivors also had immunoparalysis. When she was treated with GM-CSF, her immunoparalysis resolved, only to recur when the GM-CSF was stopped. At autopsy, she was found to have persistent *Staphylococcus Aureus* and unrecognized *Penicillium* lung infections as well as MAS.

Macrophage Activation Syndrome, as defined by Ravelli in sJIA patients, is associated in the majority of cases with infection and is improved with source control as well as anti-inflammatory treatments which are not too immunosuppressive.²⁹ Demirkol and colleagues in Turkey evaluated treatment strategies for sepsis associated MAS using a center cluster design and found 100% survival with treatment consisting of methylprednisolone or intravenous immunoglobulin (IVIG) and plasma exchange compared to 50% with dexamethasone and / or etoposide with plasma exchange.¹¹ Patients treated with the more immune suppressive regimen of dexamethasone and / or etoposide died of unremitting infection and sepsis induced multiple organ dysfunction.¹¹ Shakoory et al reported that the anti-inflammatory agent, interleukin-1 receptor antagonist protein (IRAP; Anakinra), improved survival in adult severe sepsis patients with features of MAS (defined by Hepatobiliary Dysfunction + Disseminated Intravascular Coagulation).³⁰ Anakinra has also

been successfully used to reverse MAS in critically ill children without increasing susceptibility to bacterial infection.³¹ Although MAS may be occurring during in Immunoparalysis because patients are unable to clear infection necrotic tissue, it is also possible that hyperinflammation related to MAS is itself a cause of Immunoparalysis. These studies suggest that therapies including methylprednisone or IVIG with plasma exchange as well as Interleukin-1 Receptor Antagonist Protein can effectively control MAS inflammation and restore immune competence without leading to deaths from infection.^{11,30,31}

Among the 'At risk' systemic inflammation categories, the second most common and second highest mortality was found in children who presented with a CRP ≥ 4.08 mg/dL and ferritin $< 1,980$ ng/mL. These children represented 50% of our sample and 25% of the deaths. An increasing CRP can indicate an increasing infection or necrotic tissue burden due to inadequate source control. Other potential causes of increasing CRP include surgery or tissue injury, pancreatitis, inflammatory bowel disease, organ rejection, Graft versus Host Disease, and lymphoma. Once these conditions are ruled out, the presence of an increasing CRP should warrant consideration of an exhaustive source control search including daily review of microbial culture identification and antimicrobial sensitivity reports, assurance of therapeutic antibiotic levels, and abscess / necrotic tissue debridement and drainage. In this regard, all of our non-survivors with CRP levels that persisted above 4.08 mg/dL had either persistent infection or necrotic tissue (e.g. pancreatitis) at autopsy.

There are several important limitations in our study. First, given the small sample size of our study and the likelihood that the relationship between CRP, ferritin and mortality is non-linear in this cohort, larger multiple center studies will be needed to determine whether the 2x2 contingency table approach has validity compared to the traditional continuous regression model for biomarker assessment of continuous variables. In addition larger multiple center studies will be needed to determine if adding CRP to Ferritin levels is of benefit in risk stratification. Larger sample sizes will also be needed to assess whether the table allows ongoing risk stratification with repeated sampling over time. It is also important to note that this contingency table only allows derivation of a model which now needs to be validated in a different population in a future study. Second, because CRP and ferritin levels do not fully increase nor decrease sooner than 24 hours, our study was specifically designed to recruit patients in the second day after onset of severe sepsis and to track twice weekly to monitor disease progression and response. Therefore, it is very likely that this contingency table approach will perform best in assessing disease risk and evolution rather than in sepsis diagnosis itself. Third, although the optimal cutoffs derived using the Youden Method add statistical clarity to our study, previous investigators have reported utility with other CRP and ferritin thresholds cutoffs.³⁻¹⁰ For example, Seattle Children's Hospital reported a stepwise risk when ferritin levels reached 1,000 ng/mL or 3,000 ng/mL whereas a Brazilian study noted increased risk at 500 ng/mL. More studies are required to determine the reason for these differences in cutoff values. It is plausible that these differences are related to the study population as there are highly immunosuppressed populations in Seattle and Pittsburgh. Alternatively, the variability may be related to resource differences as Brazil has less resources than Seattle and Pittsburgh. Fourth, CRP and ferritin are not the only biomarkers that can be used for risk assessment. We chose them in part because their ready availability in both resource rich and poor settings make their evaluation clinically and

economically feasible in PICUs across the globe. Procalcitonin is already approved by the United States Food and Drug Administration for bacterial sepsis risk outcome assessment. Further study will be needed to assess performance differences and / or synergies between our systemic inflammation mortality risk tool and procalcitonin measurements. Fifth, our study was not designed to assess physiologic instability mortality risk. Consequently, because we did not collect physiologic variables longitudinally, we are unable to assess whether this contingency table adds to PELOD2 or PEMOD performance in assessing disease evolution risk. Sixth, we used the Organ Failure Index to define the numbers of organs failing because it provides a simple integer scale with one point given for each organ failure.

Conclusion

In summary, we provide ‘proof of concept’ that monitoring threshold values of CRP and ferritin in a contingency table may be a relevant disease risk and evolution assessment strategy in children with severe sepsis. In theory, repeated measures of these markers may provide insight into underlying pathophysiology process and source control effectiveness, as well as help inform use of new / not established therapies (e.g. anakinra, IVIG) in pediatric sepsis. Because CRP reflects complement mediated inflammation induced by bacteria and / or necrotic host cells, and ferritin reflects macrophage activation, serial assessment of both biomarkers together allows clinicians to assess whether their therapeutic approach is facilitating pathogen and host necrotic cell clearance (reduces CRP below 4.08 mg/dL) while also reducing macrophage activation below its danger point (reduces ferritin below 1,980 ng/mL) or not. Future multicenter study will be needed to confirm optimal threshold values across a variety of sites and populations, as well as to assess if timely attainment of the goal of transitioning to the ‘Low risk’ systemic inflammation category leads to improved outcomes in children with severe sepsis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Receiver Operator Characteristics Curves for the continuous variables PRISM (AUC 0.669, 95% confidence interval 0.394 – 0.945), CRP at initial sampling (AUC 0.770, 95% confidence interval 0.607–0.934) and Ferritin at initial sampling (AUC 0.878, 95% confidence interval 0.751–1.000).

<div>Box A</div> <div>'Intermediate Risk'</div> <div>CRP < 4.08 mg/dL, and Ferritin $\geq$ 1,980 ng/mL</div> <div>Mortality 0/0 (0%)</div>	<div>Box B</div> <div>'High Risk'</div> <div>CRP $\geq$ 4.08 mg/dL, and Ferritin $\geq$ 1,980 ng/mL</div> <div>Mortality 6/13 (46.15%)</div>
<div>Box C</div> <div>'Low Risk'</div> <div>CRP $\leq$ 4.08 mg/dL, and Ferritin $\leq$ 1,980 ng/mL</div> <div>Mortality 0/44 (0%)</div>	<div>Box D</div> <div>'Intermediate Risk'</div> <div>CRP $\geq$ 4.08 mg/dL, and Ferritin < 1,980 ng/mL</div> <div>Mortality 2/43 (4.65%)</div>

Figure 2.

Subsequent mortality in children according to initial C-reactive protein and ferritin risk categories. Subsequent mortality in the 'At Risk' categories, combined "High Risk" and "Intermediate Risk" boxes, was greater than in the "Low Risk" category (8/56 (14.29%) vs 0/44 (0%), $p < 0.05$).

Figure 3.

Subsequent mortality among the patients who were in the PICU long enough for serial blood sampling who were in the 'At Risk' categories at first sampling (n = 39). The desired response, migration to the "Low Risk" box, was associated with reduced mortality (p < 0.05).

Figure 4.

This patient migrated from the 'Intermediate Risk' Box D (Ferritin < 1,980 ng/ mL and CRP 4.08 mg/dL) to the 'Low Risk' Box C and survived. The child never developed immunoparalysis or macrophage activation syndrome as inflammation resolved with appropriate antibiotics for bacterial pneumonia and septic shock.

Figure 5.

This patient migrated from the 'High Risk' category Box B (Ferritin 1,980 ng/mL and a CRP 4.08 mg/dL) to the 'Low Risk' Box C and survived. The child developed immunoparalysis (*ex vivo* TNF α response < 200 pg/mL) which resolved over time with immune suppressant tapering and appropriate antibiotic therapies. There was a secondary infection at day 23 which resolved with new antibiotics.

Table 1

Clinical Characteristics According to Systemic Inflammation Mortality Risk Categories

Status	Low Risk N = 45	Intermediate Risk N= 42	High Risk N = 13	Overall N = 100
Culture Positive	66.7%	83.3%	76.9%	75.0%
Bacterial	40.0%	73.8%	61.5%	57.0%
Viral	26.7%	19.0%	23.1%	23.0%
Fungal	6.7%	9.5%	15.4%	9.0%
Age mean +/- SD	5.01+/-5.36	5.08+/- 4.65	11.01+/-7.39	5.82+/-5.69
Female	35.6%	59.5%	46.2%	47.0%
Chronic Illness	53.3%	59.5%	76.9%	59.0%
Cancer	8.9%	11.9%	38.55%	14.0%
Transplant	20.0%	23.8%	46.2%	25.0%
PRISM mean +/- SD	7.87+/-6.36	11.21+/-8.76	18.69+/-10.72	10.68 +/- 8.71
Mortality	0.0%	4.8%	46.2%	8.0%
Max OFI mean +/- SD	2.13+/-1.27	2.12+/-0.94	3.85+/-1.46	2.35+/-1.30

PRISM = Pediatric Risk of Mortality score; Max OFI = Maximum Organ Failure Index

Table 2

Characteristics of the non-survivors with more than one sampling

Health and Infection Status	First Sample	First CRP (mg/dL)	First Ferritin (ng/mL)	Final Sample	Final CRP (mg/dL)	Final Ferritin (ng/mL)
OLTx with EBV, VRE, Adenovirus	Sepsis Day 2	4.08	15,000 ng/mL	Sepsis Day 6	1.73	13,540
Pancreatitis with β - hemolytic streptococcus	Sepsis Day 2	11.64	190	Sepsis Day 12	14.99	140
Infantile Pertussis, Streptococcus Pneumoniae, and Stenotrophomonas	Sepsis Day 2	4.76	1980	Sepsis Day 19	0.96	2,610
ALL with α -Streptococcus and Candida	Sepsis Day 2	25.61	15,000	Sepsis Day 26	29.63	6,020
AML/BMT with Enterococcus and Stenotrophomonas	Sepsis Day 2	26.38	2600	Sepsis Day 19	38.53	13,980
ALL with Escherichia coli	Sepsis Day 2	4.64	2700	Sepsis Day 6	4.3	15,000
MRSA and Penicillium	Sepsis Day 2	32.05	340	Sepsis Day 26	40.53	1320

CRP – c-reactive protein; OLTx – Orthotopic Liver Transplant; EBV – Epstein Barr Virus;

VRE – Vancomycin Resistant Enterococcus; ALL – Acute Lymphocytic Leukemia;

AML – Acute Myelogenous Leukemia; BMT – Bone Marrow Transplant;

RSV – Respiratory Syncytial Virus; MRSA – Methicillin Resistant Staphylococcus Aureus; mg/dL - milligrams per deciliter; ng/mL – nanograms per milliliter