A Systemic Inflammation Mortality Risk Assessment Contingency Table for Severe Sepsis*

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Objectives: We tested the hypothesis that a *C*-reactive protein and ferritin-based systemic inflammation contingency table can track mortality risk in pediatric severe sepsis.

Design: Prospective cohort study.

Setting: Tertiary PICU.

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

Interventions: Blood samples were attained on day 2 of sepsis and bi-weekly for biomarker batch analysis. A 2 × 2 contingency table using *C*-reactive protein and ferritin thresholds was developed.

Measurements and Main Results: A *C*-reactive protein 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" *C*-reactive protein greater than or equal to 4.08 mg/dL and ferritin greater than or equal to 1,980 ng/mL category (6/13 [46.15%]) compared with the "intermediate-risk" *C*-reactive protein greater than or equal to 4.08 mg/dL and ferritin less than 1,980 ng/mL or *C*-reactive protein less than 4.08 mg/dL and ferritin greater than or equal to 1,980 ng/mL categories (2/43 [4.65%]), and the "low-risk" *C*-reactive protein less than 4.08 mg/dL and ferritin less than 1,980 ng/mL category (0/44 [0%]) (odds ratio, 36.43 [95% CI, 6.16–215.21]). The high-risk category was also associated with the development of immunoparalysis (odds ratio, 4.47 [95% CI, 1.34–14.96]) and macrophage activation syndrome (odds ratio, 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 ($n = 19$) 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 *C*-reactive protein- and ferritin-based contingency table effectively assessed mortality risk. Reduction in systemic inflammation below a combined threshold *C*-reactive protein of 4.08 mg/dL and ferritin of 1,980 ng/mL appeared to be a desired response in children with severe sepsis. (*Pediatr Crit Care Med* 2017; 18:143–150)

Key Words: *C*-reactive protein; ferritin; immunoparalysis; macrophage activation syndrome; multiple organ failure

***See also p. 194.**

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Severe sepsis is a syndrome caused by infection-induced systemic inflammation. It is the leading cause of death in children under 5 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.

CRP 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). CRP 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 adults (4), 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 less than 200 ng/mL was associated with a 23% mortality, whereas those children with ferritin levels between 200 and 500 ng/mL had only a 9% mortality, supporting a protective role for ferritin (9). However, when ferritin levels were greater than 500 ng/mL, a 58% mortality was observed. These very high levels of ferritin are thought to represent macrophage activation syndrome (MAS) (12). In a study of all hospitalized children at Seattle Children's Hospital, ferritin levels greater than 1,000 ng/mL and greater than 3,000 ng/mL were associated with a stepwise increase in the risk of subsequent admission to the PICU or death over the next 5 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 (IRB) 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 Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (**Appendix 1**). 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 greater than or equal to 44 weeks gestation and less than 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 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, > 90th percentile for age), 2) tachypnea (respiratory rate, > 90th percentile for age), 3) abnormal temperature (< 36°C or > 38.5°C), and 4) abnormal WBC count (> 12,000 or < 4,000 mm^3 or > 10% immature neutrophils), plus the suspicion of infection (14). "Organ failure" was defined using the organ failure index (OFI) criteria of Doughty et al (15), which assigns a single integer for each organ failure (cardiovascular: need for cardiovascular support; pulmonary: need for mechanical ventilation support with a Pao_2/Fio_2 ratio < 300 without this support; hepatic: bilirubin > 1.0 mg/dL and alanine aminotransferase > 100 U/L; renal: creatinine > 1.0 mg/dL and oliguria < 0.05 mL/kg/hr; hematologic: thrombocytopenia < 100,000/μL and prothrombin time > 1.5 × normal; and CNS: 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 multiple organ failure (MOF) was defined by an ex vivo whole blood lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF)-α response less than 200 pg/mL after 3 days in a patient with two or more organ failures (16). MAS was defined by the presence of hepatic and hematologic failure and a ferritin level greater than 500 ng/mL (a modification of the criteria by Ravelli et al [17] for MAS). 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 (R&D, Minneapolis, MN). 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 greater than or equal to or less than cutoff value versus ferritin greater than or equal to or less than cutoff value. A priori, the CRP greater than or equal to cutoff value and ferritin greater than or equal to cutoff value box (box B) was considered the "high-risk" box, the CRP less than cutoff value and ferritin greater than or equal to cutoff value (box A) and the CRP greater than or equal to cutoff value and ferritin less than cutoff value (box D) were considered the "intermediate-risk" boxes, and the CRP less than cutoff value and ferritin less than cutoff value box (box C) was considered the "low-risk" box (**Fig. 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.

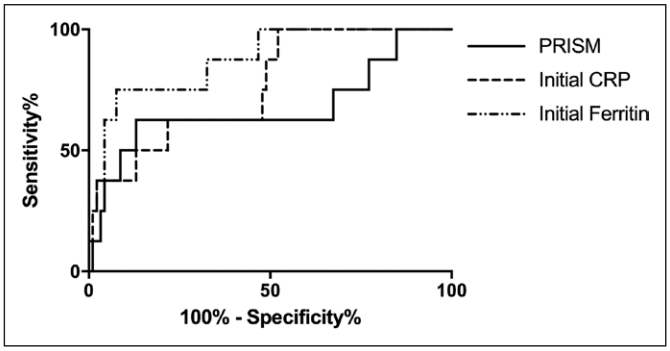

Figure 1. Receiver operator characteristic curves for the continuous variables Pediatric Risk of Mortality (PRISM) (area under the curve [AUC], 0.669; 95% CI, 0.394–0.945), *C*-reactive protein (CRP) at initial sampling (AUC, 0.770; 95% CI, 0.607–0.934), and ferritin at initial sampling (AUC, 0.878; 95% CI, 0.751–1.000).

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 with 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 MAS 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 (Pediatric Risk of Mortality [PRISM]) was analyzed using logistic regression analysis with maximum likelihood estimates and Wald chi-square and odds ratio (OR) estimates. The Youden index was used to establish optimal cutoff 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 exact test was used to test these hypotheses with p value of less than 0.05 considered significant. ORs and 95% CIs 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 of less than 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 (SAS, Cary, NC), and ROC graphs were generated using the "ROCR" package in R version 2.15.3 (https://CRAN.R-project.org/).

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 two to eight 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) (Fig. 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) (Fig. 1), and the ROC curve (n = 100) for PRISM predicting death revealed an AUC of 0.669 (95% CI, 0.394–0.945) (Fig. 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; SE, 00432; Wald chi-square, 3.1875; p = 0.0742; PRISM estimate, 0.0620; SE, 0.0412, Wald chi-square, 2.1904; p = 0.1389) with an effect OR 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; SE, 0.000238; Wald chi-square, 4.9709; p = 0.0258; PRISM estimate, 0.0273; SE, 0.0473; Wald chi-square, 0.3337; p = 0.5635) with an effect OR 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 cutoff 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 cutoff 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 greater than or equal to and less than 4.08 mg/dL and the ferritin cutoff value greater than or equal to or less than 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, ≥ 1,980 ng/mL), and high-risk (CRP, ≥ 4.08 and ferritin, ≥ 1,980 ng/mL) categories. In univariable analysis, bacterial infection (p = 0.006; Fisher exact test), age in years (p = 0.021; Kruskal-Wallis test), cancer diagnosis (p = 0.037; Fisher exact test), PRISM score (p = 0.001; Kruskal-Wallis test), maximum OFI (p < 0.001; Kruskal-Wallis test), and mortality (p < 0.001; Fisher 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, MOF, 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 (OR,

TABLE 1. Clinical Characteristics According to Systemic Inflammation Mortality Risk Categories

Status	Low Risk ($n = 45$)	Intermediate Risk ($n = 42$)	High Risk	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
Pediatric Risk of Mortality, 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
Maximum organ failure index, mean ± SD	2.13 ± 1.27	2.12 ± 0.94	3.85 ± 1.46	2.35 ± 1.30

9.58 [95% CI, 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 six of 13 (46.15%) with CRP greater than or equal to 4.08 mg/dL and ferritin greater than or equal to 1,980 ng/mL subsequently died; zero of zero (0%) with CRP less than 4.08 mg/dL and ferritin greater than 1,980 ng/mL and two of 43 (4.65%) with CRP greater than or equal to 4.08 mg/dL and ferritin less than 1,980 ng/mL died; and zero of 46 (0%) with CRP less than 4.08 mg/dL and ferritin less than 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%]) (**Fig. 3**) ($p < 0.05$). For illustrative purposes, **Figures 4** and **5** and **Supplemental Figure 1** (Supplemental Digital Content 1, http://links.lww.com/PCC/A364; legend: This patient failed to migrate from the high-risk box B [ferritin, ≥ 1,980 ng/mL and a CRP, ≥ 4.08 mg/dL] and instead, increased CRP and ferritin levels until death. The child had unremitting immunoparalysis [ex vivo TNF response, < 200 pg/mL] while receiving continued immunosuppressants [chemotherapy] and developed MAS which was not treated with IV immunoglobulin [IVIG], steroids, plasma

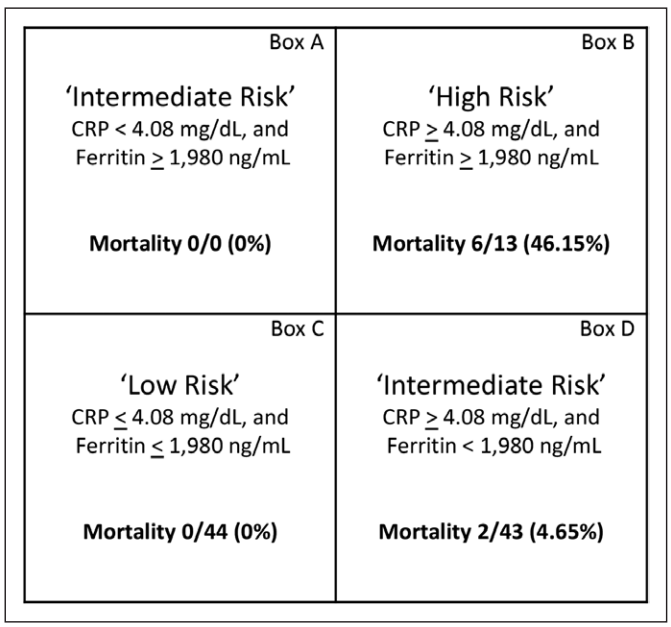

Figure 2. Subsequent mortality in children according to initial C-reactive protein (CRP) and ferritin risk categories. Subsequent mortality in the at-risk categories, combined high-risk and intermediate-risk boxes, was greater than in the low-risk category (8/56 [14.29%] vs 0/44 [0%]; $p < 0.05$).

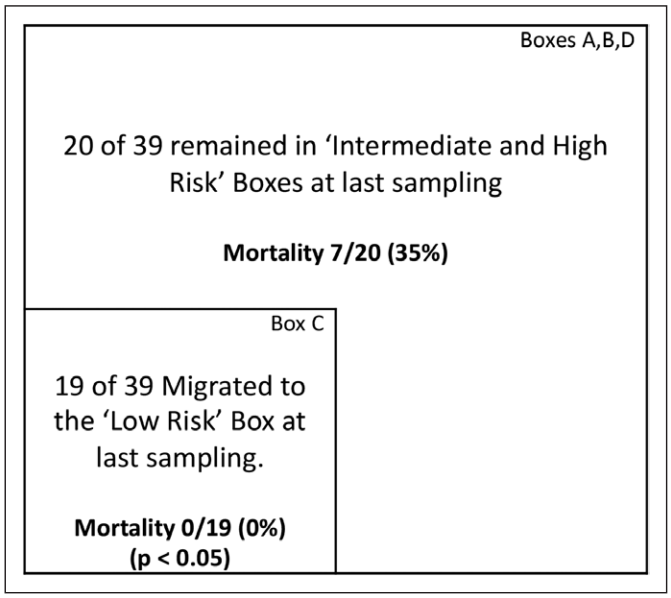

Figure 3. Subsequent mortality among the patients who were in the PICU long enough for serial blood sampling who were in the at-risk categories at first sampling ($n = 39$). The desired response, migration to the low-risk box, was associated with reduced mortality ($p < 0.05$).

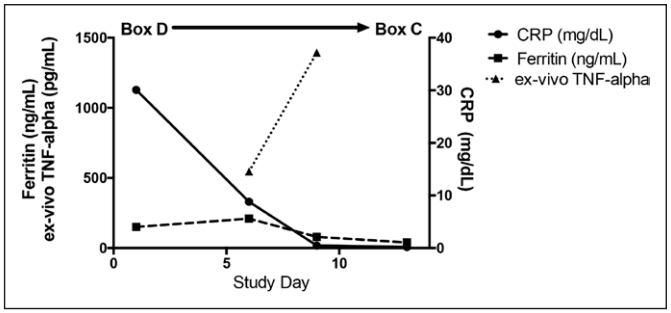

Figure 4. This patient migrated from the intermediate-risk box D (ferritin, < 1,980 ng/mL and *C*-reactive protein [CRP], ≥ 4.08 mg/dL) to the low-risk box C and survived. The child never developed immunoparalysis or macrophage activation syndrome as inflammation resolved with appropriate antibiotics for bacterial pneumonia and septic shock. TNF = tumor necrosis factor.

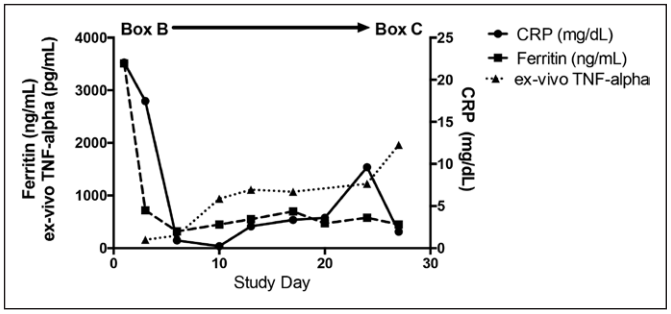

Figure 5. This patient migrated from the high-risk category box B (ferritin, ≥ 1,980 ng/mL and a *C*-reactive protein [CRP], ≥ 4.08 mg/dL) to the low-risk box C and survived. The child developed immunoparalysis (ex vivo tumor necrosis factor [TNF]-α response, < 200 pg/mL) which resolved over time with immunosuppressant tapering and appropriate antibiotic therapies. There was a secondary infection at day 23 which resolved with new antibiotics.

exchange, or anakinra) 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 nonsurvivors 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 nonsurvivors 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 greater than 4.08 mg/dL ($n = 38$) and/or a first sampling ferritin greater than 1,980 ng/mL ($n = 9$). The CRP greater than 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 greater than 1,980 ng/dL 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 CIs 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 cutoffs 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 greater than or equal to 4.08 mg/dL and ferritin greater than or equal to 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 less than 4.08 mg/dL and a ferritin greater than or equal to 1,980 ng/mL category at first sampling. A low CRP with a high ferritin can be an indicative of virus infection–associated hemophagocytosis, but our sample appears to be too small to fully evaluate this possibility.

TABLE 2. Characteristics of the Nonsurvivors 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)
Orthotopic liver transplant with Epstein-Barr virus, vancomycin-resistant Enterococcus, and 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	1,980	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
Acute myelogenous leukemia/ bone marrow transplant with Enterococcus and Stenotrophomonas	Sepsis day 2	26.38	2,600	Sepsis day 19	38.53	13,980
ALL with *Escherichia coli*	Sepsis day 2	4.64	2,700	Sepsis day 6	4.3	15,000
Methicillin-resistant *Staphylococcus aureus* and Penicillium	Sepsis day 2	32.05	340	Sepsis day 26	40.53	1,320

ALL = acute lymphocytic leukemia, CRP = *C*-reactive protein.

The next least common group was the high-risk category, a CRP greater than or equal to 4.08 mg/dL and ferritin greater than or equal to 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 immunosuppression withdrawal and to immune modulation with granulocyte-macrophage colony-stimulating factor (GM-CSF) (18–28). Among our eight nonsurvivors, five had immunoparalysis likely due to lack of tapering of chemotherapy and immunosuppressants; however, one of the previously healthy nonsurvivors 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.

MAS, as defined by Ravelli et al (17) in systemic juvenile idiopathic arthritis 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 et al (11) in Turkey evaluated treatment strategies for sepsis-associated MAS using a center cluster design and found 100% survival with treatment consisting of methylprednisolone or IVIG and plasma exchange compared with 50% with dexamethasone and/or etoposide with plasma exchange. Patients treated with the more immunosuppressive regimen of dexamethasone and/or etoposide died of unremitting infection and sepsis-induced multiple organ dysfunction (11). Shakoory et al (30) 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). 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 methylprednisolone or IVIG with plasma exchange as well as IRAP 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 greater than or equal to 4.08 mg/dL and ferritin less than 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 nonsurvivors 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 nonlinear in this cohort, larger multicenter studies will be needed to determine whether the 2 × 2 contingency table approach has validity compared with the traditional continuous regression model for biomarker assessment of continuous variables. In addition, larger multicenter 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 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 U.S. 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 Pediatric Logistic Organ Dysfunction score 2 and Pediatric Multiple Organ Dysfunction score performance in assessing disease evolution risk. Finally, we used the OFI to define the number 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 to inform the use of new/not established therapies (e.g., anakinra and 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.

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