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Performance of Interleukin-27 as a Sepsis Diagnostic Biomarker in Critically Ill Adults

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Abstract

Purpose—We recently identified interleukin-27 (IL-27) as a sepsis diagnostic biomarker in children. Here we assess IL-27 as a sepsis diagnostic biomarker in critically ill adults with systemic inflammatory response syndrome (SIRS) and sepsis.

Methods—IL-27 and procalcitonin (PCT) were measured from plasma samples in three groups: no sepsis (n = 78), pulmonary source of sepsis (n = 66), and non-pulmonary source of sepsis (n = 43). Receiver operating characteristic curves and classification and regression tree methodology were used to evaluate biomarker performance.

Results—IL-27 did not discriminate effectively between sepsis and sterile SIRS in unselected patients. The highest area under the curve (AUC) was 0.70 (95% C.I. 0.60 – 0.80) for IL-27 in subjects with a non-pulmonary source of sepsis. A decision tree incorporating IL-27, PCT, and age had an AUC of 0.79 (0.71 – 0.87) in subjects with a non-pulmonary source of sepsis. Compared to children with sepsis, adults with sepsis express less IL-27.

Conclusions—IL-27 performed overall poorly in this cohort as a sepsis diagnostic biomarker. Combining IL-27, PCT, and age reasonably estimated the risk of sepsis in subjects with a non-pulmonary source of sepsis. IL-27 may be a more reliable sepsis diagnostic biomarker in children than in adults.

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INTRODUCTION

The systemic inflammatory response syndrome (SIRS) is seen commonly in critically ill patients. SIRS is not a diagnosis, but rather a non-specific, clinical and laboratory descriptor of a generalized inflammatory state, which can occur in association with heterogeneous forms of critical illness, including sepsis (1, 2). Differentiating critically ill patients with SIRS secondary to infection (i.e. sepsis) from those with SIRS secondary to a non-infectious process (i.e. sterile inflammation) remains an important clinical challenge with therapeutic implications. Microbiologic cultures remain the diagnostic gold standard but can lack sensitivity, and there is an inherent delay between patient presentation and obtaining actionable data from such cultures. Consequently, there remains widespread interest in the development of diagnostic biomarkers that can provide an early estimation of sepsis risk in patients with SIRS, before microbiologic data become available (3–7).

Interleukin-27 (IL-27) is a heterodimeric cytokine produced by antigen presenting cells upon exposure to microbial products and inflammatory stimuli (8). IL-27 regulates T cell function and has both pro- and anti-inflammatory effects (9, 10). Ablation of IL-27 activity, by either genetic deletion or a soluble decoy receptor, confers a survival advantage in a murine model of sepsis (11). Thus, it is biologically plausible that IL-27 can serve as a sepsis diagnostic biomarker.

Using genome-wide expression profiling, we previously identified IL-27 as a candidate sepsis diagnostic gene in children with sepsis, which outperformed procalcitonin (PCT) (12, 13). We subsequently tested the diagnostic performance of IL-27 in an adult cohort and found that a combination of IL-27 and PCT identified critically ill adults with a non-pulmonary source of sepsis more reliably than either biomarker alone (14). This latter observation is consistent with the concept that sepsis diagnostic biomarkers may perform differently depending on the source of infection (15). Because biomarker performance can also depend on the population being studied, we conducted the current study to explore further the diagnostic utility of IL-27, alone and in combination with PCT, as a sepsis diagnostic biomarker in critically ill adults meeting SIRS criteria.

METHODS

Ethics statement

The study was approved by the Institutional Review Board of the University of California, San Francisco. All patients or their surrogates provided written informed consent for study participation, with the exception of (1) patients who died before they or their surrogate could be approached for informed consent and (2) patients whose critical illness precluded them from providing informed consent and for whom a surrogate could not be identified after 28 days. For these two categories of patients, the IRB approved a waiver of consent.

Study subjects and case definitions

We studied 187 prospectively enrolled critically ill adult patients admitted to either a tertiary care hospital intensive care unit (ICU) or a safety net public hospital ICU from the corresponding emergency department (as part of the Early Assessment of Renal and Lung

Injury Study) (16). Patients were excluded if they were admitted for an isolated neurological or neurosurgical diagnosis without any significant medical comorbidities or if they were admitted to the trauma service. Plasma specimens were obtained as soon as possible after presentation to the emergency department.

For this study, we selected from the cohort described above patients who met criteria for SIRS at the time of ICU admission. These patients were categorized as no sepsis (n=78); pulmonary source of sepsis (n = 66); or non-pulmonary source of sepsis (n = 43). Sepsis was defined by an attending physician after careful review of the patient's entire hospitalization, using consensus criteria (1). The source of infection was similarly determined by attending physician review, as in prior studies (16–18). The classification of a pulmonary source of sepsis was based on a combination of radiographic data (chest roentgenogram or chest computed tomography), microbiologic data (sputum or bronchoalveolar lavage samples), and impression of the treating physician team.

Measurement of IL-27 and PCT plasma concentrations

IL-27 (EMD Millipore Corporation, Billerica, MA, USA) and procalcitonin (Bio-Rad, Hercules, CA, USA) protein concentrations were measured in duplicated plasma samples using a magnetic bead multiplex platform and a Luminex 100/200 System (Luminex Corporation, Austin, TX, USA), according to the manufacturers' specifications. These were the same assays used in our original pediatric study (13), and we have not observed any differences in assay performance between the two study periods.

Initially, biomarker data were described using medians, interquartile ranges, and percentages. Biomarker comparisons between groups used the Mann-Whitney U-test (SigmaStat Software, Systat Software, Inc., San Jose, CA). Receiver operating characteristic (ROC) curves and the respective area under the curve (AUC) were constructed and compared using SigmaStat Software. Associations between IL-27 concentrations and selected clinical variables were measured using simple linear regression.

Classification and regression tree (CART) analysis was conducted using the Salford Predictive Modeler v6.6 (Salford Systems, San Diego, CA) (13, 19, 20). The primary outcome variable for the modeling procedures is sepsis. The secondary outcome variable for the modeling procedure is sepsis from a non-pulmonary source of infection, as in our previous report (14). The CART procedure considered IL-27, PCT, and age as potential predictor variables. Weighting of cases and the addition of cost for misclassification were not used in the modeling procedures. Performance of the derived model is reported using diagnostic test statistics with 95% confidence intervals computed using the score method as implemented by the VassarStats Website for Statistical Computation (21).

RESULTS

Primary Analysis

Table 1 provides the clinical characteristics of the study subjects. Compared to the subjects without sepsis, a greater proportion of subjects with a pulmonary source of sepsis met the SIRS temperature criterion and a greater proportion of subjects with a non-pulmonary

source of sepsis met the SIRS white blood cell criterion. Subjects with a pulmonary source of sepsis were also more likely to have an oncologic comorbidity compared to the subjects without sepsis. Subjects in both sepsis groups met a greater number of overall SIRS criteria compared to subjects without sepsis. Among the subjects with sepsis, those with a non-pulmonary source of sepsis were more likely to have a positive culture compared to those with a pulmonary source of sepsis. No other differences were observed.

Plasma samples were obtained at a median of 9.5 hours (range: 10 minutes to 32 hours) from the time of admission to the Emergency Department. Neither IL-27 nor PCT were effective at discriminating between patients with SIRS alone and patients with SIRS due to sepsis in the overall cohort, with an AUC of 0.58 (95% C.I. 0.50 – 0.66) for IL-27 and an AUC of 0.62 (0.54 – 0.70) for PCT.

Based on our prior findings that the performance of IL-27 in adult patients may vary based on the source of sepsis (14), we then analyzed the performance of IL-27 in patients stratified by pulmonary versus non-pulmonary sepsis. Median plasma IL-27 concentrations were higher in the subjects with a non-pulmonary source of sepsis, compared to subjects without sepsis and subjects with a pulmonary source of sepsis (Table 2). Likewise, median plasma PCT concentrations were higher in the subjects with a non-pulmonary source of sepsis compared to subjects without sepsis. In subjects with a pulmonary source of sepsis, the AUCs for the IL-27 and PCT ROC curves both had 95% confidence intervals that included the line of no discrimination (i.e. an AUC = 0.5; Table 2). In subjects with a non-pulmonary source of sepsis, the AUCs were significantly greater than the line of no discrimination, but the overall performance of each individual biomarker for estimating the risk of sepsis was marginal.

In a *post hoc* analysis, we tested the performance of IL-27 and PCT in subjects with sepsis and a laboratory-confirmed infection (n = 51) exclusively. When comparing these subjects to the subjects without sepsis, the AUC for IL-27 was 0.70 (0.61 – 0.79) and the AUC for PCT was 0.69 (0.60 – 0.78). We also conducted a *post hoc* analysis to determine if IL-27 concentrations were influenced by the type of infecting pathogens. The median IL-27 concentration (IQR) in subjects with a gram-positive bacterial infection (n = 17) was 3.5 ng/ml (2.6 – 5.7), and in subjects with a gram-negative bacterial infection (n = 25) it was 3.3 ng/ml (2.6 – 4.7) (p = 0.768). Finally, we conducted *post hoc* analyses to determine if IL-27 concentrations were associated with illness severity, the time lag between Emergency Department admission and sample collection, or gender. By simple linear regression, IL-27 concentrations were not associated with APACHE II scores ($r^2 = 0.013$; p = 0.141), APACHE III scores ($r^2 = 0.010$; p = 0.178), or sample collection lag time ($r^2 = 0.003$; p = 0.490). The median IL-27 serum concentration in females (n = 94) was 2.8 ng/ml (1.7 – 3.5), and in males (n = 91) it was 2.6 ng/ml (1.8 – 3.8) (p = 0.964).

Secondary Considerations

We recently demonstrated that predictor variables that perform poorly in isolation could be combined in a decision tree to substantially improve predictive capacity (22). Because both IL-27 and PCT alone had limited ability to discriminate sepsis from SIRS in this adult cohort, and based on our previous study in critically ill adults with sepsis (14), we next

derived a decision tree incorporating IL-27, PCT, and age as potential predictor variables for estimating the risk of non-pulmonary sepsis in patients with SIRS. Age was incorporated based on prior data showing that the performance of diagnostic biomarkers in sepsis may vary by age (23). Figure 1 shows the derived tree, which contains three low sepsis probability terminal nodes (TN1, TN2, and TN4; sepsis probability 0.0 to 0.16), two intermediate sepsis probability terminal nodes (TN3 and TN5; sepsis probability 0.40 to 0.63), and one high sepsis probability terminal node (TN6; sepsis probability 0.87). The diagnostic test characteristics (95% C.I.) of the decision tree are as follows: sensitivity 86% (71 – 94); specificity 56% (45 – 67); positive predictive value 52% (40 – 64); negative predictive value 88% (75 – 95); positive likelihood ratio 2.0 (1.5 – 2.6); and negative likelihood ratio 0.2 (0.1 – 0.5). As shown in Figure 2, the AUC (95% CI) of the decision tree 0.79 (0.71 – 0.87) was significantly greater than that of either IL-27 or PCT alone.

Although the derived decision tree had an AUC approaching 0.8, the performance of IL-27 in this adult cohort was overall inferior to what we observed previously in critically ill children (13). Accordingly, we compared the IL-27 plasma concentrations of the current adult cohort to that of our previously published pediatric cohort. As shown in Figure 3, critically ill children with sepsis have a significantly higher median IL-27 concentration compared to adults with sepsis, and the interquartile ranges differ by a factor of at least 3.

DISCUSSION

In the primary analysis, neither IL-27 nor PCT alone reliably estimated the risk of sepsis in this cohort of critically ill adults with SIRS. However, in the secondary analysis a decision tree incorporating rules based on IL-27, PCT, and age reasonably estimated the risk of sepsis in subjects with a non-pulmonary source of sepsis, which is consistent with our previous study in critically ill adults (14). Previous biomarker studies have also demonstrated differences in biomarker performance depending on the source of infection (15), but the biological basis of the discrepancy in the performance of IL-27 between non-pulmonary versus pulmonary causes of sepsis is not evident.

In our previous study involving critically ill children with heterogeneous sources of sepsis, IL-27 alone had a >90% specificity and positive predictive value for identifying sepsis, with an AUC that was significantly greater than that of PCT alone (13). We have not been able to replicate these results in either our previous study of critically ill adults (14), nor in the current study. Thus, our studies so far indicate that IL-27 may be a more reliable sepsis diagnostic biomarker in critically ill children than in critically ill adults. Additional studies in pediatric populations are currently under way, but our current, direct comparison of IL-27 concentrations in children and adults supports this assertion. This comparison indicates that children with sepsis on average have higher plasma levels of IL-27, compared to their adult counterparts. It may be that the developing host has a greater capacity to express IL-27 in response to infection, and that this greater dynamic range increases the utility of IL-27 as a sepsis diagnostic biomarker in children. Whether increased plasma levels of IL-27 reflect increased production by dendritic cells, decreased turnover, and/or differences in cellular binding is not known. Nonetheless, the concept that developmental age influences the host response to infection (24–27), as well as sepsis diagnostic biomarker performance (23), is

now well supported in the literature. The observation that age contributed to the decision tree in the current study further supports the concept that IL-27 production during sepsis may be, in part, age dependent.

We note important differences in case definitions between our three studies thus far. In the original study involving children with SIRS, all patients with sepsis had a positive, laboratory-confirmed bacterial culture from either blood or other normally sterile body fluid (13), which was not the case in either adult study. In our subsequent adult study, subjects were enrolled consecutively, upon admission to the intensive care unit, and the subjects without sepsis did not necessarily meet criteria for SIRS (3, 14). In the current study, all subjects met criteria for SIRS, but a positive culture was not required for the sepsis classification; this study design was chosen to be the most reflective of the typical situation encountered by the practicing physician needing to estimate the risk of sepsis in a critically ill patient with SIRS. Thus, differences in case definitions could account for some of the differences between our three studies, and these important distinctions illustrate how biomarker performance can depend heavily on the population being studied.

We note the following limitations of the current study. The number of patients with a non-pulmonary source of infection was relatively small, thus it is possible that the diagnostic accuracy of IL-27 is under- or overestimated in the current study. CART methodology has the potential to over fit data, thus the validity of the decision tree needs to be tested in an independent cohort. In our previous study in adults, we generated an IL-27- and PCT-based decision tree having an AUC of 0.92 (14), but that tree could not be tested in the current cohort because none of the subjects in the current cohort had PCT values below the primary PCT-based decision rule. This likely reflects the fact that our two adult studies used different PCT assays. Thus, it is imperative that future studies use comparable assays.

In conclusion, IL-27 alone performed poorly in this cohort of critically ill adults with SIRS. It appears that IL-27 has greater utility as a sepsis diagnostic biomarker in critically ill children than in critically ill adults. The biological basis of this finding may include a greater capacity of the developing host to express IL-27 in the setting of infection. Our current study suggests that a combination of IL-27 and PCT has the potential to reasonably estimate the risk of sepsis in critically ill adults with a non-pulmonary source of sepsis. Additional studies are currently under way in pediatric and adult populations to assess these observations further.

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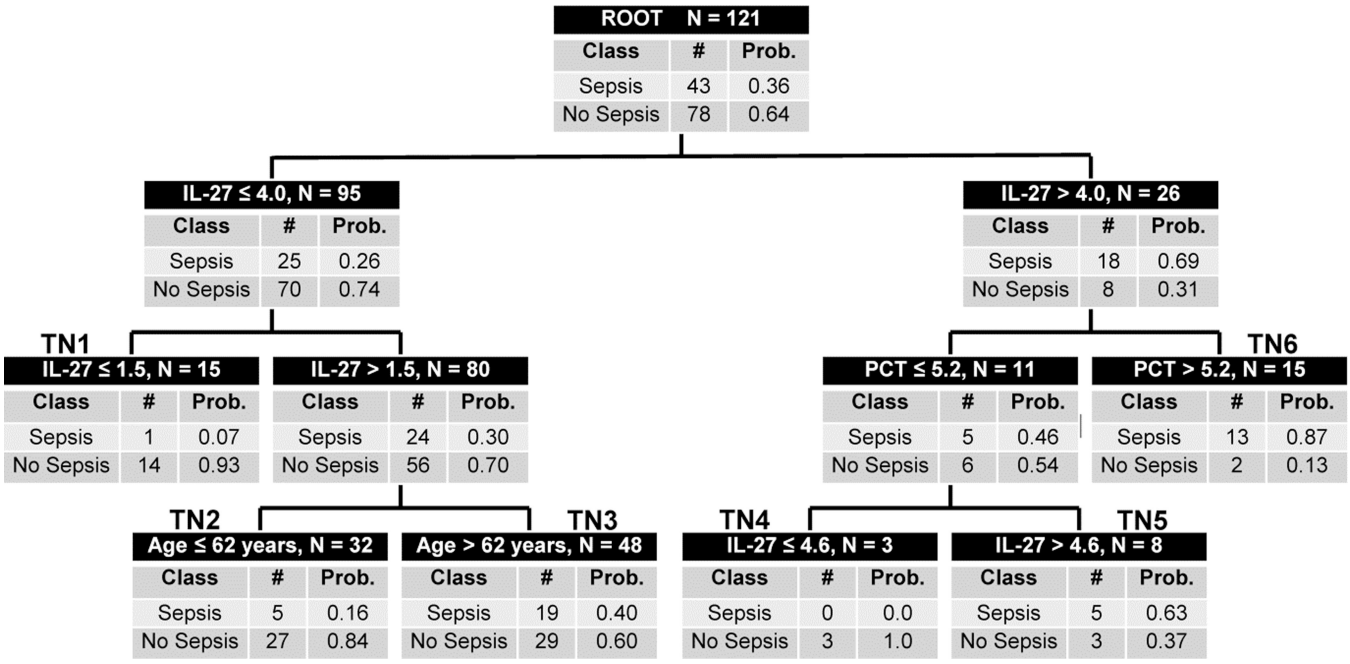

Figure 1.

The CART-derived decision tree for estimating the probability of sepsis in subjects with a non-pulmonary source of infection, based on IL-27, PCT, and age. Each node provides the total number of subjects in the node, the IL-27-, PCT-, or age-based decision rule, and the number of patients with and without sepsis, with the respective probabilities. Terminal nodes (TN) 1, 2, and 4 are considered low sepsis probability nodes, terminal nodes 3 and 4 are considered intermediate sepsis probability nodes, and terminal node 6 is considered a high sepsis probability node. To calculate the diagnostic test characteristics, all subjects in the low probability terminal nodes (n = 50) were classified as predicted no sepsis, whereas all subjects in the intermediate and high probability terminal nodes (n = 71) were classified as predicted sepsis.

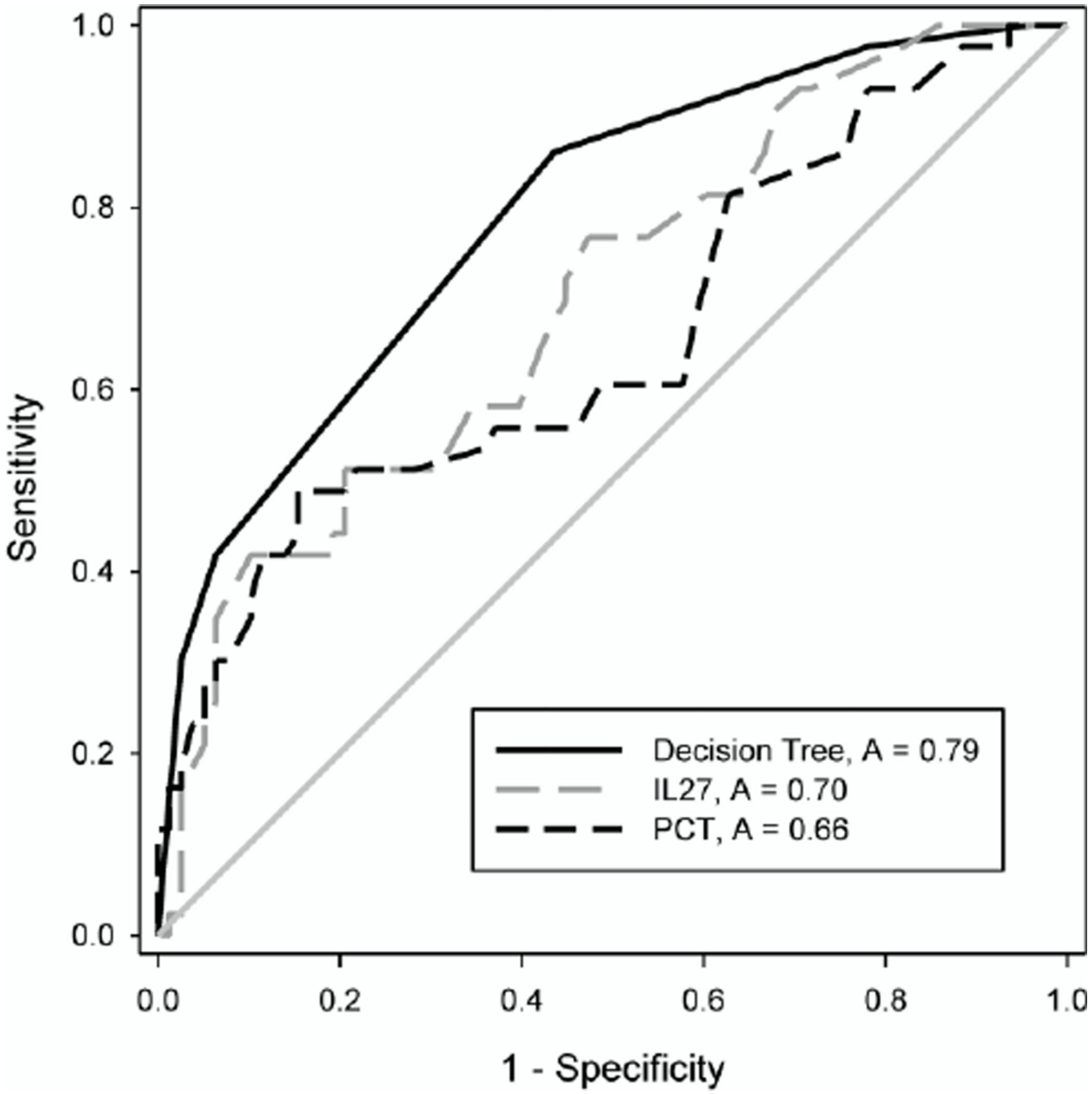

Figure 2.

ROCs for the decision tree, IL-27 alone, and PCT alone in subjects with a non-lung source of sepsis. The AUC for the decision tree was significantly greater than the AUCs for IL-27 alone and PCT alone ($p < 0.05$).

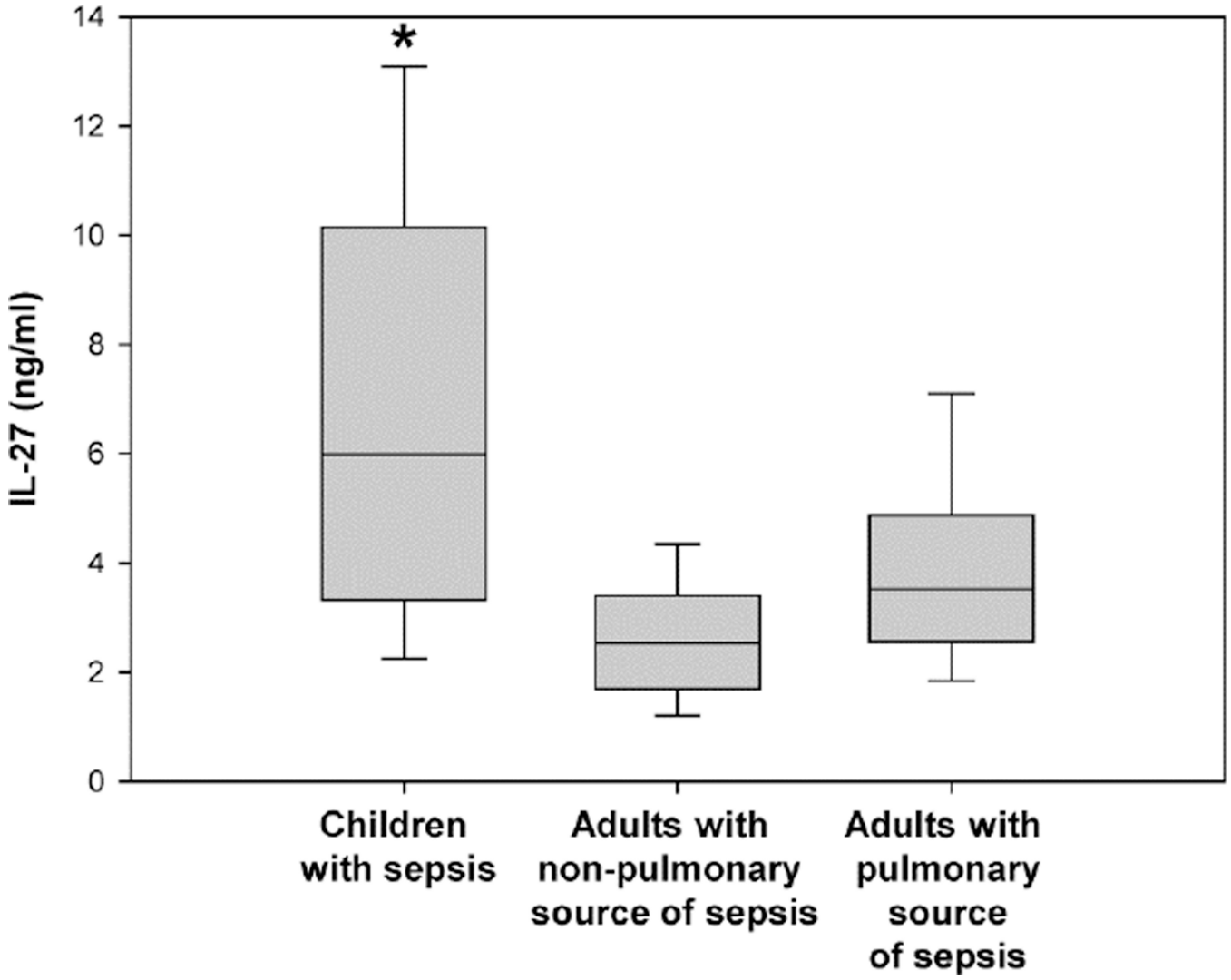

Figure 3.

Box-whisker plots for IL-27 concentrations in our previously published pediatric cohort (n = 130, reference #13), and the current cohort of adults with pulmonary (n = 66) and non-pulmonary (n = 43) sources of sepsis, respectively. *p < 0.05 vs. adults with a non-pulmonary source of sepsis and adults with a pulmonary source of sepsis, ANOVA on Ranks.

Table 1

Characteristics of the study subjects.

	No Sepsis	Sepsis Pulmonary Source	Sepsis Non-Pulmonary Source
N	78	66	43
Median Age, Years (IQR)	62 (50 – 75)	65 (50 – 78)	65 (51 – 80)
Median APACHE III score (IQR)	76 (53 – 112)	87 (65 – 117)	87 (58 – 109)
Race, number (%)			
Caucasian	40 (51)	33 (50)	23 (53)
African-American	17 (22)	13 (20)	4 (9)
Asian	18 (23)	17 (26)	14 (33)
Other	3 (4)	3 (5)	2 (5)
Major comorbidities, number (%)			
Cardiovascular	54 (69)	49 (74)	27 (63)
Pulmonary	10 (13)	17 (26)	6 (14)
Hepatic	9 (12)	2 (3)	1 (2)
Renal	15 (19)	10 (15)	9 (21)
Diabetes	26 (33)	18 (27)	11 (26)
Oncologic	13 (17)	23 (35)[1]	13 (30)
Immunosuppression	6 (8)	10 (15)	7 (16)
Chronic steroids	7 (9)	6 (9)	6 (14)
Number (%) meeting individual SIRS criteria			
Temperature	31 (40)	45 (68)[1]	23 (53)
Heart rate	71 (91)	59 (89)	41 (95)
Respiratory rate	66 (85)	58 (88)	37 (86)
White blood cell count	34 (44)	39 (59)	30 (70)[1]
Median Number of SIRS criteria met (IQR)	2 (2 – 3)	3 (2 – 4)[1]	3 (2 – 4)[1]
Number with positive cultures (%)[3]	--	22 (33)	29 (67)[2]
Number with negative cultures (%)	--	32 (48)	9 (21)[2]
Number with indeterminate cultures (%)	--	12 (18)	5 (12)

[1] $P < 0.05$ vs. No Sepsis.

[2] $P < 0.05$ vs. Pulmonary source of sepsis.

[3] Seventeen subjects (33%) had infection secondary to gram-negative bacteria, 25 subjects (49%) had infection secondary to gram-positive bacteria, and the remaining 9 subjects (18%) had either mixed bacterial infection, viral infection, or *Plasmodium falciparum*.

Table 2

IL-27 and PCT data.

	No Sepsis	Sepsis Pulmonary Source	Sepsis Non-Pulmonary Source
Median IL-27 ng/ml[1]	2.5 (1.6 – 3.4)	2.5 (1.7 – 3.4)	3.5 (2.6 – 4.9)[2]
Median PCT ng/ml	4.4 (3.8 – 5.2)	5.0 (3.9 – 6.0)	5.3 (4.1 – 7.4)[3]
AUC-ROC for IL-27	--	0.50 (0.40 – 0.60)	0.70 (0.60 – 0.80)
AUC-ROC for PCT	--	0.60 (0.50 – 0.69)	0.66 (0.55 – 0.76)

[1] Median IL-27 concentrations in normal healthy children (reference #13): 1.0 ng/ml (0.7 – 1.6)

[2] $P < 0.05$ vs. No Sepsis and Pulmonary Source of Sepsis.

[3] $P < 0.05$ vs. No Sepsis.