I, William J Hanna, hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
IL-27: A Novel Biomarker in Predicting Bacterial Infection Among the Critically Ill

Student's name: William J Hanna

This work and its defense approved by:

Committee chair: Erin Nicole Haynes, Dr.P.H.

Committee member: Jennifer M Kaplan, M.D.

Committee member: Hector Wong, M.D.
IL-27: A Novel Biomarker in Predicting Bacterial Infection Among the Critically Ill

A thesis submitted to the

Graduate School

of the University of Cincinnati

in partial fulfillment of the

requirements for the degree of

Master of Science

in Clinical & Translational Research

In the Department of Environmental Health

Division of Epidemiology & Biostatistics

of the College of Medicine

April, 2015

by

William Joseph Hanna

MD, University of Kentucky, 2007

BA/BS, Marshall University 2001

Committee Chair: Erin N. Haynes, PhD
Abstract:

Introduction and Objective: Distinguishing bacterial infections from non-infectious conditions through use of rapid and accurate biomarkers remains a diagnostic challenge in critically ill patients. Available biomarkers, such as procalcitonin, continue to exhibit inconsistent results. Interleukin 27 (IL-27), a heterodimeric cytokine produced by antigen presenting cells, has shown early promise in critically ill children, exhibiting high specificity and positive predictive values for bacterial infection in a recent preliminary study. Given the need for more effective biomarkers in bacterial sepsis, the early promise shown by IL-27, and the dearth of other studies investigating its value for this purpose, we hypothesize that IL-27 may effectively serve as a diagnostic biomarker for bacterial infection in the critically ill patients and propose the following study to further investigate its value in this context.

Methods: Eligibility criteria included those subjects with a clinical suspicion of infection, defined through the acquisition of blood cultures by the primary PICU team. Blood samples collected within 6 hours of blood culture acquisition were retrieved through waiver of informed consent and used for both IL-27 and procalcitonin (PCT) biomarker assay testing. Values for bacterially infected patients, defined using both microbiologic and clinical data, were then compared to values for patients with no evidence of infection as judged by an intensivist chart review blinded to biomarker results. Formal performance comparisons included calculations of ROC curves for IL-27 and PCT individually in addition to a combination strategy utilizing an optimal decision tree generated by CART analysis. Secondary analysis was then performed using a definition of bacterial infection focused upon subjects with documented bloodstream infections.

Results: 410 patients were enrolled, with an infection rate of 27%. Patient ages ranged from 2 months to 34 years, with median=9.5 yrs, and IQR 2.9-15.7 yrs. ROC curves for the primary analysis yielded IL-27 and PCT AUCs of 0.61 (0.54 to 0.67) and 0.65 (0.59 to 0.71) respectively. Secondary analysis with the modified definition yielded AUCs of 0.76 (0.67 to 0.84) and 0.72 (0.64 to 0.81) respectively, with a specificity of 95% (92%,97%) for the prior established cut-point value of ≥ 5.0 ng/ml. Among immune compromised patients, IL-27 and PCT AUCs were 0.82 (0.72 to 0.93) and 0.73 (0.60 to 0.86), and a CART derived decision tree incorporating both yielded an AUC of 0.89 (0.83 to 0.95).

Conclusion: These data and analyses are part of a larger study of IL-27 and PCT, with a planned enrollment of 700 patients. Consistent with the preliminary study, our data suggests that IL-27 may serve as a useful biomarker in estimating risk of bacterial infection among critically ill patients with blood stream infections. Among the cohort of immune compromised patients in particular, its predictive value appears promising, with a combination IL-27 and PCT strategy yielding a significantly improved performance compared to PCT alone.
Acknowledgements

I would like to respectfully acknowledge members of my Scholarship Oversight Committee for their guidance in the formation and constant revision of this study.

Scholarly Oversight Committee: Hector Wong, MD, Lesley Doughty, MD, Erika Stalets, MD, Jenny Kaplan MD, Erin Haynes, Ph.D.

I would like to thank Drs. Lesley Doughty, Erika Stalets, Jenny Kaplan for their support and encouragement throughout. Thanks to Dr. Erin Haynes for being a great teacher and advisor. Thanks to Patrick Lahni and Kelli Harmon for their help in running samples and orienting me during my time in the laboratory. Thanks also to the CCHMC laboratory for their willingness to regularly retrieve samples for analysis.

I would especially like to thank Dr. Hector Wong for mentoring me throughout the duration of the study, including the generation and revision of the study design, the interpretation and analysis of results, and the reviewing and revising of presentations related to the study.
Table of Contents

I. List of table and figures vi
II. List of abbreviations vii
III. IL-27: A Novel Biomarker in Predicting Bacterial Infection Among the Critically Ill
   A. Introduction 1
   B. Methods 2
   C. Results 4
   D. Discussion 4
IV. References 6
V. Appendices
   A. Tables and Figures 8
Tables and Figures

Table 1: Demographic Characteristics of Both Groups

Table 2: Test Characteristics of Determining Bacterial Infection

Figure 1: Schema of Overall Study Design

Figure 2: ROC Curves for Bacteremic vs. Uninfected Patients

Figure 3: CART Analysis Combining both Procalcitonin and IL-27 Among the Immune Compromised

Figure 4: ROC Curve for Bacteremic vs. Uninfected Patients Among the Immune Compromised
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-27</td>
<td>Interleukin 27</td>
</tr>
<tr>
<td>PCT</td>
<td>Procalcitonin</td>
</tr>
<tr>
<td>CCHMC</td>
<td>Cincinnati Children’s Hospital Medical Center</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operative Characteristic</td>
</tr>
<tr>
<td>PICU</td>
<td>Pediatric Intensive Care Unit</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
</tr>
</tbody>
</table>
Introduction:

The Systemic Inflammatory Response Syndrome (SIRS) is a generic term used to describe patients with fever and other signs of systemic inflammation. SIRS can be a non-specific manifestation of multiple clinical entities NOT associated with infection, or it can be a manifestation of systemic infection (i.e. sepsis). The ability to distinguish sepsis from SIRS is important, as sepsis, with an estimated mortality of 1.6 million infants and children per year worldwide, is thought to be the leading cause of death in this cohort. In the United States, > 75,000 cases of pediatric severe sepsis are estimated annually with an in-hospital mortality of roughly 14.4% [1-4].

Sepsis may occur as a result of any number of microorganisms (viruses, bacteria, fungi, etc.). While the gold standard in diagnosing sepsis remains positive microbiological cultures, delays from time of acquisition to final result have led many investigators to explore the use of sepsis biomarkers as a faster and more effective diagnostic strategy. In this context, recognizing bacterial forms of sepsis early is critical because it warrants prompt antibiotic therapy, and it has been demonstrated that delays in antibiotic administration in patients with bacterial sepsis have negative consequences for outcome. Thus, using bacteria specific biomarkers for earlier detection, and therefore more timely and appropriate treatment, could prove of great benefit in decreasing both mortality and morbidity related to bacterial sepsis [5-8].

Among current biomarkers used in sepsis, procalcitonin remains one of the more utilized, despite having variability in performance depending on patient population and with a recent metaanalysis in adults showing lack of reliability in distinguishing septic from uninfected patients in critically ill cohorts. Other biomarkers continue to be tested however, with a recent study suggesting early promise for IL-27, a heterodimeric cytokine produced by antigen presenting cells [9-20].

Using a large genome wide expression database of critically ill children admitted to pediatric intensive care units across the United States, 100 class predictor genes, differentially expressed between patients with and without bacterial sepsis, were isolated using computer-assisted image analysis of gene arrays. Of these 100 predictor genes, EBI3, a subunit of IL-27, was discovered as having the highest predictive strength for bacterial infection. Both IL-27 and procalcitonin concentrations were then measured within a cohort of 231 critically ill children. Findings included a specificity and positive predictive value of >90% for bacterial infection in those with IL-27 levels ≥ 5ng/ml, performing significantly better than procalcitonin [18].

Given this, the dearth of other studies investigating the value of IL-27 for this purpose, and the need for more effective biomarkers in bacterial sepsis, we hypothesize that IL-27 can effectively serve as a diagnostic biomarker among the critically ill population, and propose the following validation study to further investigate the value of this novel candidate biomarker in this context.
Methods

Following Institutional Review Board approval, we performed a prospective cohort study of patients admitted to the Cincinnati Children’s Hospital Medical Center (CCHMC) PICU with suspected infection between April of 2013 and December 2014.

Inclusion Criteria

Two criteria were required for study eligibility. The first was admission to the PICU with clinical suspicion for infection. This clinical suspicion was strictly defined by the acquisition of a blood culture at any point during admission by the primary ICU team, performed independently and without interference from the research team. This pragmatic approach captures the exact context in which a diagnostic biomarker would be used by clinicians (i.e. patients with a clinical suspicion of bacterial infection), one that may lead to more generalizable results as compared to a study based on more stringently selected patients. The second was the availability of a residual blood sample within 6 hours of blood culture acquisition, obtained via waiver of informed consent.

Exclusion Criteria

No exclusion criteria were utilized.

Study Procedures

Using the CCHMC electronic medical record, the investigating team was notified daily of all blood cultures sent within the prior 24 hours in the intensive care unit. It was then determined which of these patients had a residual, otherwise to be discarded, serum sample within this same time frame in the CCHMC clinical laboratory. These samples were obtained from the lab and used to measure both IL-27 and procalcitonin levels using the magnetic bead multi-plex platform (EMD Milipore Corporation, Billerica, MA) and Luminex 100/200 System (Luminex Corporation, Austin, TX). These measurements required <100 μl of serum in total. See figure 1 for schema of study design.

Determination of Bacterial Infection

Bacterial infection was defined using both laboratory and clinical data. Patients designated as “infected” included all patients with clinically relevant positive bacterial microbiological cultures collected within 48 hours of enrollment. For the primary analysis, these cultures included blood, urine, cerebrospinal, pleural, peritoneal, stool, wound, and endotracheal/tracheal tube cultures. Of note, those patients with strong evidence for bacterial infection in the absence of positive cultures were also included in the
“infected” designation. These cases included such findings as radiographic evidence (CT scan, CXR, etc.) or physical exam findings strongly suggesting bacterial infection in the absence of positive cultures. All other subjects were classified as “non-infected”. Given concerns of misclassification bias (i.e. misclassifying “uninfected” controls as “infected” cases), a secondary analysis was performed designating only patients with culture positive blood infections as “infected” and those with no clinical suspicion as uninfected.

Data Collection

In addition to the IL-27 and PCT measurements, relevant demographic data collected included age, gender, reason for admission, type of admission (surgical vs. medical), presence of comorbidities, evidence of preexisting immune suppression, and both source and etiology of infection. Evidence of immune suppression was determined by an intensivist chart review blinded to biomarker results and utilizing data such as use of chronic immunosuppressive medications and evidence of conditions commonly associated with immune-dysregulation.

Sample Size Calculation

Using data from the previously published preliminary study, we proposed a sample size 700 ICU patients. A main goal of this is to estimate the precision of specificity and sensitivity estimates for IL-27 as a biomarker for bacterial infection. Assuming an expected prevalence of approximately 20% and a specificity of 92% as reflected in our preliminary study, 700 patients would result in 95% CI for the estimate of specificity of ± 2%. The same sample size will also give a 95% CI of ± 8% for the sensitivity of 62%. Given the above preliminary data, we anticipated data collection to span roughly 20 months.

Because procalcitonin is currently being used clinically as a diagnostic biomarker for bacterial infection, the primary analysis also included a comparison of IL-27 performance to PCT performance, using calculations of respective ROC curves. For comparing the AUC for IL-27 to that for PCT, assuming areas of about 0.80 and 0.75 as found in our preliminary studies, 700 patients were calculated to provide 90% power to find the difference, assuming an α = 0.05 and a prevalence of bacterial infection of 20%.

Statistical Analysis

Utilizing SigmaStat Software (Systat Software Inc., San Jose, CA, USA), all continuous variables were presented as median values and categorical variables as percentages. Statistical tests used to compare study cohorts included Pearson Chi Square and Mann Whitney Rank Sum Tests. Receiver Operating Characteristic (ROC) curve analysis was also performed for both IL-27 and PCT. Test characteristics and their respective 95% confidence intervals (CI) were calculated using diagnostic test statistics provided by VassarStats Website for Statistical Computation. Classification and Regression Tree (CART) analysis (Salford Predictive Modeler v6.6) was utilized in the secondary analysis. CART analysis is computer
generated prediction tool that utilizes outcome variables of interest to generate a decision making tree whose predictive value can then be tested.

Results

Demographics

A total of 410 patients have been analyzed to date. These patients have a median age of 9.5 (IQR 2.9, 15.7) years. Twenty seven percent (N=110) of the included patients were determined to be infected, 77% of which had positive cultures and 23% negative cultures. Among those with positive cultures, 48% were isolated from the blood, 21% from the respiratory tract (including samples from endotracheal aspirates and bronchoalveolar lavage), and 19% from the urinary tract. See Table 1 for further demographic characteristics.

Diagnostic Accuracy

Differences in median values between infected and uninfected groups were significant for both IL-27 (1.8 vs. 1.3 ng/ml, p <0.001) and procalcitonin (6.3 vs. 5.6 ng/ml, p < 0.001). ROC curves generated resulted in AUCs of 0.61 (0.60, 0.73; p<0.001) for IL-27 and 0.65 (0.54, 0.68; p<0.001) for PCT. The area difference between the AUCs was not significant. See figure 2 and table 2 for ROC curve and associated test characteristics.

Secondary Analysis

Given concerns of misclassifying “uninfected” controls as “infected” cases, a secondary analysis was performed utilizing a definition of infection that included only patients with clinically relevant positive blood cultures. Three hundred and forty three patients were included, 45 (13%) of which were classified as infected. ROC curves yielded AUCs of 0.76 (0.67-0.84) for IL-27 and 0.72 (0.64 to 0.81) for procalcitonin, with an area difference of 0.04 (p=0.40) (figure 2). Using this definition and the prior cut point of 5 ng/ml for IL-27, test characteristics included a specificity of 95% and a PPV of 47% (39-66). See table 2 for full test characteristics at different cut off points.

Among the subclass of patients classified as immune suppressed, ROC curves revealed AUCs of 0.82 (0.70-0.93) and 0.73 (0.60-0.87) for IL-27 and PCT respectively. A subsequent Classification and Regression Tree (CART) Analysis was performed on this subclass. Starting with the “root” node that included all patients (N=133) and dividing into 2 subsequent or “daughter” nodes, four terminal nodes were ultimately generated, each with specific cut off points for IL-27 and PCT (see figure 4). Terminal node 1 was designated as a lower risk node with none (0%) of the 73 patients being infected. Terminal nodes 2, 3, and 4 were designated as higher risk nodes compared to the “root” node, with rates of 13%, 17%, and 50% respectively. Using these designations, the CART algorithm was then tested, yielding an AUC of 0.89 (0.83, 0.95) which was significantly improved from PCT alone (p=0.02) (figure 4). Test characteristics for the tree yielded a sensitivity of 100% (77-100), specificity of 63% (53-75), NPV of 94-100) of 100%, and PPV of 28% (18-42).
Discussion

In the primary analysis, both IL-27 and procalcitonin demonstrated poor reliability for estimating bacterial infection risk. A trend to improvement was noted however when a secondary analysis was performed utilizing a modified definition of infection that included only those patients found to be bacteremic. With this definition, an IL-27 cut off value of ≥5 ng/ml was noted to have a similar diagnostic value as a “rule in” test to the original study, exhibiting a specificity of 95%. The lower PPV may be explained by a lower prevalence of positive cases, as the prior study exhibited a prevalence of positive cases nearly five times (56%) the current cohort [20]. Among those classified as immune compromised, a continued improved trend in predictive value was found, with the test characteristics of the CART analysis indicating high sensitivity and NPV.

The results of the secondary analysis are consistent with recent adult studies showing improved predictive values of IL-27 when excluding pulmonary sources of infection from the ‘infected’ definition [21-22]. Given that recent population-based studies indicate a bacterial etiology in only roughly 8% of children diagnosed clinically and radiographically with pneumonia, concerns of misclassification bias may be even more heightened among such populations as these [23]. By only classifying patients with clinically relevant culture positive bloodstream infections as ‘infected’ and using those with no concerns for infection as a comparator, this bias may have been minimized. Misclassification aside, given that the majority of infections diagnosed in the original pediatric study were isolated from culture positive bloodstream infections, our results may also suggest IL-27 to have greater predictive value in bacteremic patients compared to infections from other body compartments [20,22].

Although conclusions are limited by a relatively small number of infected patients, the continued improved predictive trend of IL-27 among the subset of immunocompromised patients is a noteworthy finding. Of note, >80% of those patients designated as immune compromised were being administered immunosuppressive medications and roughly 50% had a diagnosis requiring bone marrow or solid organ transplantation. When combining both IL-27 and PCT in a CART generated algorithm, the sensitivities and negative predictive values of 100% suggest the combination may serve as a potent “rule out” test for this very difficult-to-manage population of patients. If this trend continues to exist following full enrollment (N=700), such results may only warrant further validation studies but also basic research investigations, as the immunobiological mechanism of IL-27 in this context appears unclear. While IL-27, produced by antigen presenting cells upon stimulation by microbial products, might be thought of as less predictive among populations in which such cells are compromised, such a perspective likely greatly simplifies the picture. Both the complex pleotropic nature of IL-27 in addition to the vast variety of immunopathology combined under the heading of “immune compromised” would warrant further elucidation [18,24].

Notable limitations to our study include the continued concern of misclassification bias. In order to further minimize this, a consensus among 3 intensivists blinded to biomarker results will be used to classify patients for the final analysis of 700 patients. Second, the accuracy of results in the secondary
analysis may be over or underestimated, given the relatively small numbers of patients included. This will be partly addressed following complete patient enrollment. Third, given the relatively novel use of IL-27 as a biomarker in this context, temporal production of IL-27 in the context of infection is still poorly understood and will need to be focused upon in subsequent studies. Finally, severity of illness scoring was not included in the analysis as a potential confounder. This will also be addressed following completion of full patient enrollment.

In conclusion, our results suggest that Interleukin 27 may serve as a useful biomarker in estimating risk of bacterial infection among critically ill patients with blood stream infections. In particular, among those classified as immune compromised, a combination strategy utilizing both IL-27 and procalcitonin may aid in “ruling-out” bacterial infection when a clinical suspicion for infection arises. These results currently have several limitations however, which will be largely addressed prior to the full study completion of 700 patients.

References


Table 1: Demographic Characteristics of Both Groups

<table>
<thead>
<tr>
<th></th>
<th>Infected (27%)</th>
<th>Uninfected (73%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.6 (IQR 2.8-17.8)</td>
<td>7.3 (IQR 2.9-14.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>Male (%)</td>
<td>57%</td>
<td>60%</td>
<td>0.83</td>
</tr>
<tr>
<td>Immune suppressed state</td>
<td>29%</td>
<td>38%</td>
<td>0.06</td>
</tr>
<tr>
<td>Post-operative admission</td>
<td>3%</td>
<td>13%</td>
<td>0.004</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>70%</td>
<td>73%</td>
<td>0.80</td>
</tr>
<tr>
<td>IL-27 levels (median)</td>
<td>1.8 (1.0-3.2)</td>
<td>1.3 (0.8-2.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCT levels (median)</td>
<td>6.3 (5.5-7.7)</td>
<td>5.6 (5.0-6.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 2. ROC curves for Infected vs. Uninfected Patients

ROC Curves

N= 410
Infected = 110 (27%)
Uninfected = 300

95% CI
PCT (0.54 to 0.68)
IL-27 (0.60 to 0.73)

Table 2. IL-27 Test Characteristics for predicting bacterial infection

<table>
<thead>
<tr>
<th>IL-27 (ng/dL)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>48%(37-58)</td>
<td>72%(66-76)</td>
<td>78%(73-83)</td>
<td>57%(29-48)</td>
</tr>
<tr>
<td>3.0</td>
<td>23%(20-36)</td>
<td>89%(85-92)</td>
<td>77%(72-81)</td>
<td>50%(37-62)</td>
</tr>
<tr>
<td>4.0</td>
<td>20%(13-28)</td>
<td>93%(89-95)</td>
<td>76%(71-82)</td>
<td>52%(37-57)</td>
</tr>
<tr>
<td>5.0</td>
<td>12%(6-19)</td>
<td>95%(90-97)</td>
<td>74%(70-79)</td>
<td>43%(26-52)</td>
</tr>
<tr>
<td>6.0</td>
<td>10%(5-17)</td>
<td>95%(93-98)</td>
<td>74%(70-79)</td>
<td>48%(27-58)</td>
</tr>
</tbody>
</table>
Figure 3. ROC Curves for Bacteremic vs. Uninfected Patients

ROC Curves

N = 343
Infected = 43 (12.5%)
Uninfected = 300

95% CI
PCT (0.64 to 0.81)
IL-27 (0.67 to 0.84)

Table 3. IL-27 Test Characteristics for Predicting Bacterial Infection in Secondary Analysis

<table>
<thead>
<tr>
<th>IL-27 (ng/dL)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>72% (55-85)</td>
<td>71% (63-81)</td>
<td>94% (86-99)</td>
<td>14% (10-46)</td>
</tr>
<tr>
<td>3.0</td>
<td>51% (42-73)</td>
<td>89% (82-98)</td>
<td>95% (84-96)</td>
<td>44% (23-62)</td>
</tr>
<tr>
<td>4.0</td>
<td>37% (27-67)</td>
<td>93% (89-97)</td>
<td>91% (84-94)</td>
<td>52% (32-68)</td>
</tr>
<tr>
<td>5.0</td>
<td>26% (12-48)</td>
<td>95% (90-98)</td>
<td>90% (80-94)</td>
<td>47% (39-66)</td>
</tr>
<tr>
<td>6.0</td>
<td>21% (9-44)</td>
<td>96% (93-99)</td>
<td>89% (79-93)</td>
<td>50% (40-67)</td>
</tr>
</tbody>
</table>
Figure 4. Classification and Regression Tree (CART) combining IL-27 and procalcitonin for the prediction of bacterial infection in immune suppressed critically ill patients. “Infected” denotes bacteremic cases and “Uninfected” denotes those with no clinical suspicion of bacterial infection. Four terminal nodes were generated having variable risks of infection.

Figure 5. ROC curves for immune suppressed patients and bacteremic infection. Included in green is the ROC curve for the CART analysis performed.

N = 133
Infected = 17 (13%)
Uninfected = 116