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Biochemical and Hematological Markers of Inflammation Accurately Predict Sepsis and its Severity in ICU Patients

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Sepsis has long been considered a major health crisis, striking more than 1.5 million Americans each year. [1] It is also one of the leading causes of death in hospitalized patients, killing more than 250,000 people annually in the U.S. [2], equating to one person every two minutes. The complex pathobiology and expansive reach to most organ systems in the body make sepsis an extremely dangerous disease. Considering the severity of sepsis and new regulatory requirements, rapid diagnosis and treatment has become a major area of focus for emergency department and Intensive Care Unit clinicians. As a result, researchers have turned to investigating groups of clinical tests to effectively diagnose or predict the onset of sepsis.

A study performed at Vanderbilt University Medical Center (VUMC) examined the use of hematologic parameters (including WBC, RBC, platelets, ANC and IG), procalcitonin (PCT), and CRP for predicting sepsis among SIRS patients. Receiver operator characteristic (ROC) curves were generated to evaluate various sepsis diagnostic models using the hematological and biomarker results collected one or two days prior to the patient developing symptoms of systemic inflammation. The area under the ROC curve (AUC) when using only hematology parameters to predict sepsis ranged from 0.51 to 0.66. When looking at procalcitonin and C-reactive protein only, the AUC increased to 0.70 and 0.73, respectively. When the absolute neutrophil count (ANC) and IG count were combined with the inflammatory biomarkers, the AUC increased to 0.74, showing good predictive ability to identify sepsis in patients before the onset of systemic inflammatory symptoms. Additionally, when trying to predict which patients would develop severe sepsis or septic shock, this same combination of hematologic and inflammatory biomarkers gave an AUC of 0.77. The VUMC study, current literature, and changing guidelines demonstrate that the bedside physical examination along with laboratory testing (to include hematologic and inflammatory biomarkers) are the most effective combination of parameters that clinicians currently have to rapidly and accurately predict or diagnose sepsis in a critically ill patient.

Key Words Sepsis, Systemic Inflammatory Response Syndrome, SIRS, Inflammation, Intensive Care Unit, Immature Neutrophils, Immature Granulocyte Count, IGC, IG, Automated Differential, Hematology, White Blood Cell, Sysmex.

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INTRODUCTION & BACKGROUND

Sepsis has long been considered a major health crisis, striking more than 1.5 million Americans each year. [3] It is also one of the leading causes of death in hospitalized patients, killing more than 250,000 people annually in the U.S. [4], equating to one person every two minutes. Rapid diagnosis of sepsis is of utmost importance; a study by Kumar et al. shows that mortality increases approximately 8% for each hour treatment is delayed in patients with septic shock. [5] Despite increased vigilance, sepsis is on the rise in the U.S. Factors such as antibiotic resistance and increasing numbers of immunocompromised and aging patients will likely lead to additional sepsis diagnoses in the future and, with that, higher medical expenditures. In 2013, septicemia was the most expensive illness to treat, costing $23.7 billion and accounting for 6.2% of all dollars spent on inpatient care. [6] And while overall hospital costs remained fairly stable, treatment expenses for sepsis rose by 19% over the two prior years. [2]

The complex pathobiology and expansive reach to most organ systems in the body make sepsis an extremely dangerous disease. Sepsis begins with the introduction of an invading organism – bacterial, viral, fungal, or parasitic – which triggers an immune response. Cell injury caused

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by the invading pathogen initiates release of prostaglandins (which starts the inflammatory process) and cytokines (chemicals that summon cells of the immune system to the site of infection). The acute inflammatory response is essential to the success of the innate immune system and is, in itself, a complex process.

Many changes take place at the beginning of the inflammatory process. To start, the microvasculature surrounding the infected area dilates, allowing increased blood flow. Gaps form between the cells in the tissue surrounding the inflamed area; immune cells such as neutrophils and macrophages pass through these gaps and migrate to the affected areas more easily. Increased circulation to the site also promotes healing of the damaged cells and delivery of activated coagulation factors.

In most cases the localized response of the innate immune system, assisted by the cell-mediated or acquired immune system, is sufficient to control and overpower the infectious agent and restore the health of the patient. However, sometimes this physiologic response is impaired, resulting in more harm than good. Normally, both pro and anti-inflammatory mediators are released in response to an infectious agent to ultimately ensure return to homeostasis. When this process is not in balance and the infectious agent and/or inflammatory markers are unchecked, endothelial cell dysfunction can progress to tissue injury, then to organ dysfunction, and eventually to death (***Fig. 1***).

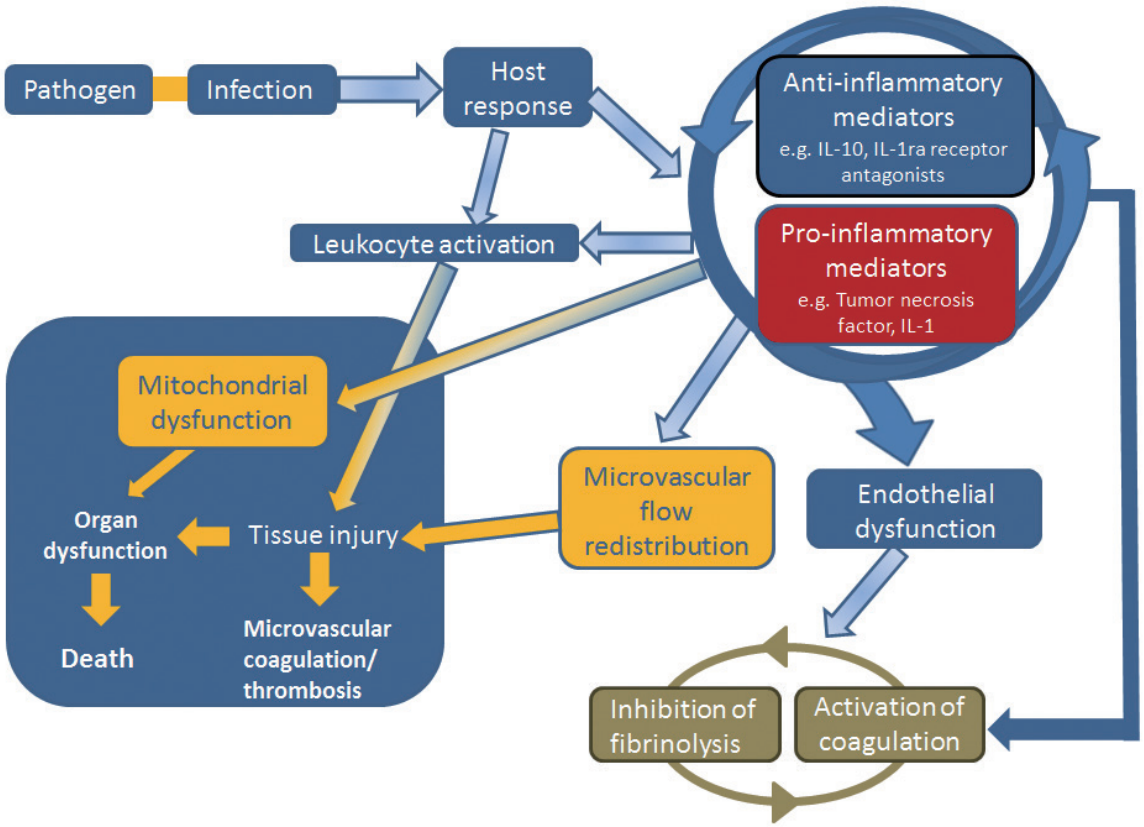

Fig. 1 *Woodworth, A. Systemic Inflammatory Response → Coagulation Activation → Impaired Fibrinolysis → End organ dysfunction → Hypotension. http://media.aacc.org/shows/pearls/10-04-17_Woodworth_Final/presentation.html*

Considering the severity of sepsis and new regulatory requirements, rapid diagnosis and treatment has become a major area of focus for emergency department and Intensive Care Unit clinicians. In 1992, the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) collaborated to develop recommendations for diagnosis and treatment of sepsis.[7] These guidelines were refined in 2004 under the auspices of the Surviving Sepsis Campaign [8] which was formed by a partnership of the SCCM, the European Society of Intensive Care Medicine (ESICM), and the International Sepsis Forum. [9] The goal of this campaign was to publish a standardized definition of sepsis with the aim of improving patient outcomes through rapid diagnosis and prompt treatment. The task force determined three levels of severity within the sepsis diagnosis – sepsis, severe sepsis, and septic shock – with increasing organ system involvement and coinciding mortality rates. They also defined the Systemic Inflammatory Response Syndrome (SIRS) as a condition where at least two of the four following criteria have been met: temperature > 38° or < 36°C; a WBC count > 12,000 or < 4,000/μL; heart rate in excess of 90 beats/minute; and respiratory rate > 20 breaths/minute.

Although SIRS can result from a variety of insults upon the human body including ischemia, burns, trauma, and infection [10], SIRS specifically induced by an infectious agent (as defined by a positive blood or body fluid culture) was defined as sepsis. Though this classification became known as the "gold standard" for sepsis diagnosis, it was not without its limitations: a) cultures can require multiple days to detect the presence of a pathogen; b) cultures are subject to contamination, resulting in both false positive and false negative findings; and c) cultures may be negative in as many as 50% of septic patients.[11] Therefore, the sepsis definition was restructured to include SIRS with documented or suspected infection.

The most recent sepsis definition, released in February 2016 by SCCM/ESICM as Sepsis-3, defined sepsis as a "life-threatening organ dysfunction caused by a dysregulated host response to infection." [12] In these guidelines, the committee acknowledged that sepsis is a complicated disease and that "no current clinical measures reflect the concept of a dysregulated host response." Identification of this condition, however, may be aided by the Sequential Organ Failure Assessment (SOFA) scoring system which is a clinical tool for evaluating sepsis and its severity in patients (***Fig. 2***). The SOFA score incorporates six physiologic systems (respiratory, coagulation, hepatic, cardiovascular, renal and neurological) and assigns points based on the degree of organ system dysfunction. A SOFA score of ≥ 2 and a documented or suspected infection is considered sepsis. This scoring system, as well as its abbreviated assessment tool, the Quick SOFA (q-SOFA) designed for use with emergency department and ambulatory patients, is intended to properly identify patients with sepsis or septic shock. Even though these newer guidelines and tools changed the definition and diagnosis of sepsis, they are only applicable to patients well into the disease process and are still reliant upon the presence (or suspected presence) of infection.

System	Score				
	0	1	2	3	4
Respiration **PaO_2/FiO_2 mmHg**	≥ 400	< 400	< 300	< 200 and mechanical ventilation	< 100 and mechanical ventilation
Coagulation **Platelets x 10^3**	≥ 150	< 150	< 100	< 50	< 20
Liver **Bilirubin (mg/dl) [μmol/L]**	< 1.2 [<20]	1.2-1.9 [20-32]	2.0-5.9 [33-101]	6.0-11.9 [102-204]	> 12.0 [> 204]
Cardiovascular **Mean Arterial Pressure or vasopressors required**	≥70 mm/Hg	<70 mm/Hg	Dopamine ≤ 5 μg/kg/min or dobutamine (any dose)	Dopamine ≤ 5 μg/kg/min or epinephrine ≤ 0.1 μg/kg/min or norepinephrine ≤ 0.1 μg/kg/min	Dopamine ≤ 15 μg/kg/min or epinephrine > 0.1 μg/kg/min or norepinephrine > 0.1 μg/kg/min
Central Nervous System **Glasgow Coma Scale score**	15	13-14	10-12	6-9	<6
Renal **Creatinine (mg/dl) [μmol/L] or Urine output**	< 1.2 [< 110]	1.2-1.9 [110-170]	2.0-3.4 [171-299]	3.5-4.9 [300-440] < 500 ml/day	> 5.0 [> 440] < 200/day

Fig. 2 *SOFA scoring guidelines. Adapted from Vincent et al.* [13]

This leads to the problematic task of reliably identifying an infection, especially in the absence of positive culture results. To this end, many studies have attempted to identify biomarkers that can accurately differentiate between patients with infectious (sepsis) and non-infectious causes of SIRS. This is where laboratory testing outside of microbiology steps onto the stage. Although hundreds of biomarkers have been studied, only a few are robust enough for routine clinical use in sepsis management. To complicate testing matters further, thus far no single biomarker is able to reliably identify sepsis in patients with SIRS. As a result, researchers have turned to investigating groups of clinical tests to effectively diagnose or predict the onset of sepsis.

One of the biomarkers often used is the White Blood Cell (WBC) differential, which is used to determine the type and relative amount of white cells present in a blood sample. Enumerating the various subsets of leukocytes in a patient's peripheral blood is an invaluable tool for detecting various disorders and conditions. In the case of infection, neutrophils play a very important role in the immune response by releasing cytokines that attract macrophages and by phagocytizing cellular debris. An increased absolute neutrophil count (ANC) is a common finding in an infectious or inflammatory condition [14]. In untreated infections or infections where the treatment is ineffective, hyperproliferation of neutrophils often occurs in the bone marrow, resulting in immature granulocytes being released into the peripheral blood. These immature neutrophilic cells are further subclassified as follows: non-segmented (band) forms, metamyelocytes, myelocytes, promyelocytes and myeloblasts (though blast forms are rarely seen in the peripheral blood during infection). The presence of immature granulocytes outside of the bone marrow is commonly termed a "left shift" and may be considered a possible indicator of infection. While the traditional laboratory definition of left shift includes all stages of immature granulocytes, the importance of band enumeration has been challenged with the advent of automated cell counting methods. According to current literature, band counts provide limited clinical utility and may not be recommended for diagnosis of infection. [15]

To standardize the WBC differential and increase laboratory efficiency, hematology testing platforms are now equipped with automated cell counters that effectively differentiate the five mature cell types (neutrophil, lymphocyte, monocyte, eosinophil, and basophil) and identify when immature granulocytes are present. When immature cells are flagged by the analyzer, a blood smear must be reviewed to identify and quantitate the cell types present. Historically, this time consuming and subjective process was manually performed by a skilled laboratorian. The standard procedure requires that only 100 cells are counted in a blood film that likely contains thousands. Furthermore, inconsistency in sample preparation (poor mixing, poor distribution, smear too thick or too thin, etc.) and lack of manual differential precision leave many clinicians wishing for a more reproducible and reliable measurement of immature granulocytes. [16]

Analyzers capable of performing a so-called "6-part" automated differential are able to quantitate Immature Granulocytes (IG). Studies verifying the reliability of automated IG counts (which include only metamyelocytes, myelocytes and promyelocytes) conclude that this parameter is accurate even when immature granulocytes are present in very small numbers in the peripheral blood, and that the automated IG count is a valid substitution for the traditional manual differential.[17] Moreover, it has been reported that the IG count is a useful screening parameter for acute infection, surpassing the predictive value of the WBC count, and compared to bands is a better predictor of infection when the WBC count is normal. [10,18] Therefore, bands provide little added benefit [19] and it has been noted that "laboratorians can reasonably advocate for removal of this criterion from clinical decision support tools."[20] Additional studies have found that an increased automated IG count can significantly discriminate between infected and non-infected patients and displays the highest discriminative power for infection within the first 48 hours after the onset of SIRS when compared to other biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6) and lipopolysaccharide binding protein (LBP). [21] Furthermore, the automated IG offers advantages over microscopy by counting more cells than the standard 100-cell differential, markedly reduces statistical error by standardizing the identification of immature cell types, and performs similar to the band count for identification of infection. [22,23] As a result, the recommendation of the College of American Pathologists is that laboratories discontinue reporting bands as an individual cell type. [24]

MATERIALS & METHODS

As no single contemporary biomarker can reliably identify sepsis, clinicians utilize a combination of biomarkers and clinical parameters (***Fig. 3***). Because the automated 6-part WBC differential carries the advantages of low cost and shorter turnaround time, the IG count may play a beneficial role in developing new diagnostic algorithms for identifying sepsis in ICU patients. [25] A study performed at Vanderbilt University Medical Center (VUMC) examined the use of hematologic parameters (including WBC, RBC, platelets, ANC and IG), procalcitonin (PCT), and CRP for predicting sepsis among SIRS patients. The study group used a software program to scan electronic medical records of ICU patients, aimed at identifying those exhibiting symptoms of SIRS. The software flagged patients with ≥ 2 SIRS criteria recorded in a 24 hour period. During the study, residual plasma specimens from 210 patients sent to the clinical laboratory for testing on the day SIRS was diagnosed (Day 0), as well as one and/or two days prior to the date of diagnosis (Day -1 and Day -2, respectively) were retrieved. The previously run hematological parameters were recorded for Days 0, -1, and -2 and specimens from these days were analyzed for PCT and CRP (***Fig. 4***).

All records were submitted to two different Medical Intensive Care Unit (MICU) physicians in order to adjudicate the sepsis diagnosis. The physicians agreed on the diagnosis of 200 out of 210 patients (96%) originally identified by the electronic alert. Of those 200 patients, 70 were excluded from the study because of lack of Day -1 or Day -2 samples for retrospective biomarker analysis; the remaining samples were separated into 60 patients with

Infection (documented or suspected) and some of these parameters:

General variables
- Fever or hypothermia
- Heart rate >90/min^{-1} or >2SD above normal
- Tachypnea
- Altered mental status
- Edema or positive fluid balance
- Hyperglycemia (glucose >140 mg/dL (7.7 mmol/L) in a non-diabetic

Organ dysfunction variables
- Acute oliguria
- Ileus
- Arterial hypoxemia (Pao2/Fio2<300)
- Creatinine increase >0.5 mg/dL (44.2 umol/L)
- Coagulation abnormalities (INR>1.5 or aPTT >60s)
- Thrombocytopenia (platelet count <100x10^3 uL^{-1})
- Hyperbilirubinemia (total, >4 mg/dL (70 umol/L))

Inflammatory variables
- Leukocytosis or leukopenia (WBC count >12x10^3 or <4x10^3 uL^{-1}, respectively)
- >10% immature cells
- C-reactive protein >2SD above normal value
- Procalcitonin >2SD above normal value

Hemodynamic variables
- Arterial hypotension

Tissue perfusion variables
- Hyperlactatemia >1mmol/L
- Decreased capillary refill or mottling

Fig. 3 *Diagnostic Parameters associated with Sepsis. Critical Care Medicine. 41(2); February 2013*

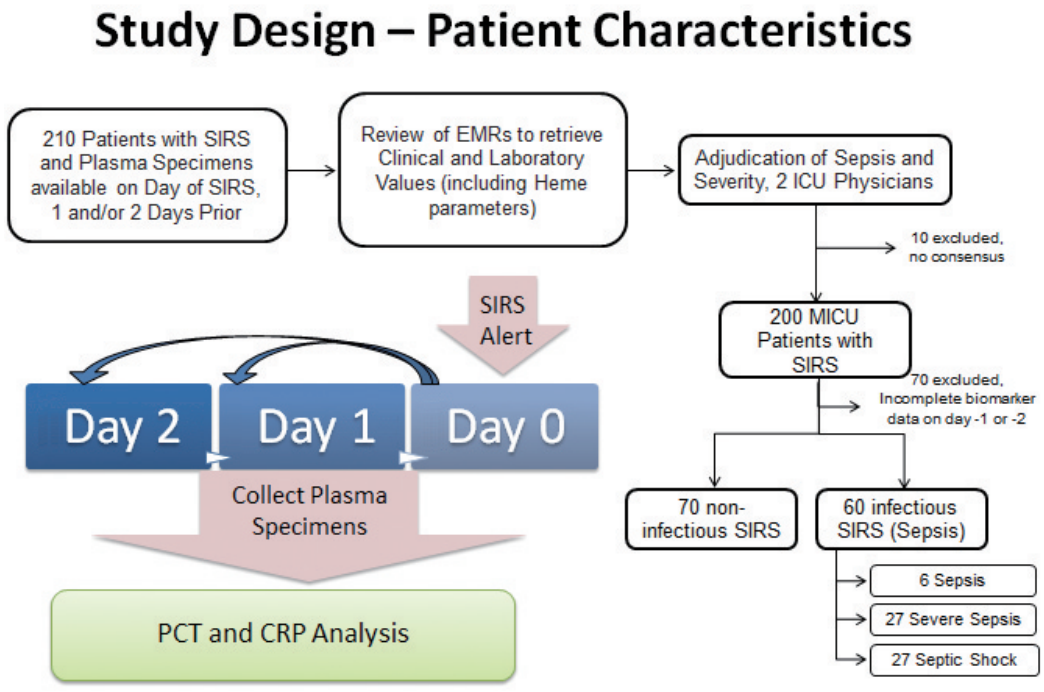

Fig. 4 *Diagnostic Utility of Biomarker Models to Predict Severe Sepsis/Shock (VUMC study design)*

infection (sepsis) and 70 without infection (SIRS). Almost half (n = 27) of the patients diagnosed with sepsis were identified as having septic shock, the most critical form of sepsis, with another 27 patients having severe sepsis.

RESULTS & DISCUSSION

Receiver operator characteristic (ROC) curves were generated to evaluate various sepsis diagnostic models using the hematological and biomarker results collected one or two days prior to the patient developing symptoms of systemic inflammation (***Table 1***). The area under the ROC curve (AUC) when using only hematology parameters to predict sepsis ranged from 0.51 to 0.66. When looking at procalcitonin and C-reactive protein only, the AUC increased to 0.70 and 0.73, respectively. When the absolute neutrophil count (ANC) and IG count were combined with the inflammatory biomarkers, the AUC increased to 0.74, showing good predictive ability to identify sepsis in patients before the onset of systemic inflammatory symptoms. Additionally, when trying to predict which patients would develop severe sepsis or septic shock, this same combination of hematologic and inflammatory biomarkers gave an AUC of 0.77 (***Table 2***).

Table 1 *Areas under the ROC curves were generated for individual biomarkers and hematological parameters to predict which patients would progress to sepsis 1 or 2 days prior to patients developing SIRS. If samples were available for both days -1 and -2, the maximum pre-SIRS concentration was utilized for all analytes except platelets.*

	Prediction of Sepsis			Prediction of Severe Sepsis/Shock		
Pre-SIRS Predictor	**AUC**	**95% CI**	**P value**	**AUC**	**95% CI**	**P value**
Max Procalcitonin (PCT)	0.70	[0.61-0.79]	<0.0001	0.73	[0.63-0.82]	<0.001
Max C-reactive Protein (CRP)	0.73	[0.64-0.82]	<0.0001	0.74	[0.66-0.83]	<0.001
Max White Blood Cell Count (WBC)	0.66	[0.56-0.75]	0.002	0.64	[0.54-0.74]	0.007
Min WBC	0.63	[0.53-0.73]	0.010	0.61	[0.51-0.71]	0.030
Max Immature Granulocyte Count (IGC)	0.59	[0.49-0.69]	NS	0.60	[0.50-0.70]	0.056
Max Absolute Neutrophil Count (ANC)	0.66	[0.56-0.75]	0.002	0.64	[0.55-0.74]	0.005
Min Platelet Count	0.51	[0.41-0.61]	NS	0.51	[0.40-0.61]	NS
Red Blood Cell Count (RBC)	0.51	[0.41-0.61]	NS	0.53	[0.43-0.63]	NS

Table 2 *Area under the curve values for several models to predict patients that would develop severe sepsis or septic shock (Sepsis — 3 definition) before patients met SIRS criteria in the MICU.*

Pre-SIRS Predictor	AUC	P value
ANC and IG	0.65	0.014
PCT and CRP	0.74	<0.001
All Heme - Leukocytosis	0.67	0.027
All Heme - Leukopenia	0.67	0.027
ANC + IG + PCT + CRP	0.77	<0.001

CONCLUSION

Patients with sepsis often present with nonspecific symptoms of inflammation which rapidly progress to a more severe condition if not treated. In uncontrolled cases of sepsis, acute organ dysfunction and shock may develop. Mortality rates in patients with septic shock exceed 50%. [26] Because of this rapid progression, it is of utmost importance that patients be diagnosed and treated in a timely fashion. The VUMC study, current literature, and changing guidelines demonstrate that the bedside physical examination along with laboratory testing (to include hematologic and inflammatory biomarkers) are the most effective combination of parameters that clinicians currently have to rapidly and accurately predict or diagnose sepsis in a critically ill patient.

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