The Economical and Clinical Value of Rapid Diagnosis in Sepsis

Nicole Lynn Gentile

Writer's Comment: *Having seen a close friend of mine suffer through his rigorous Ph.D. qualifying exam on sepsis, I wanted to contribute to his suffering by learning more about the subject at hand and asking him tough questions. Through simple literature searches, exposure to ongoing sepsis research, and consultation with clinicians, I was able to derive a broad yet detailed picture of important elements contributing to mortality associated with sepsis. Ironically, little did I know that this research paper would inspire me to pursue a masters degree—potentially requiring me to defend in sepsis as well. I can only hope that writing this paper has provided me with a sufficient foundation to help further my knowledge of sepsis, while also enhancing my future capacity to be a clinician scientist. I would like to dedicate this paper to the Kost Lab in the Department of Pathology at UCD. You all work very hard and I am honored to be a part of the "family."*

—Nicole Lynn Gentile

Instructor's Comment: *Nicole Gentile's piece, "The Economical and Clinical Value of Rapid Diagnosis in Sepsis," offers proof (positive) that scientific writing–in the right hands–can be a work of art and a thing of beauty. While the topic of Nicole's research might seem–at first–to be monoscopic given its base argument that "Sepsis with acute organ dysfunction (severe sepsis) is the most common cause of death in non-coronary ICU patients," it turns out that her advantageous use of seamless prose, impeccable source notation, organizational composure, and savvy use of entrance/exit strategies–sharp opening + smart closure– reminds us, one and all, that even in the otherwise confined space of a research article for a UWP 104F class @ UCD elegance is, and remains, the order of the day. But perhaps even more important than the sheer elegance of her work as a whole, Nicole Gentile's finished research also serves to remind us that scientific narrative, as mode*

of composition, can be every bit as compelling as the more traditional prose narratives used in the "humanities" …

—James McElroy, University Writing Program

Sepsis with acute organ dysfunction, or severe sepsis, is the most common cause of death in non-coronary ICU patients and the eleventh leading cause of death overall.[1] Angus et al. report that 750,000 Americans develop severe sepsis annually, equaling 2,000 new cases per day. The number of cases has increased 139% in the past decade and is expected to increase even further in the future. Despite advances in critical care medicine, severe sepsis mortality ranges from 28% to 50%.[1]

What is Sepsis?

According to the 1992 statement from the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM), sepsis is defined as suspected or proven infection plus systemic inflammatory response syndrome (SIRS).[2] SIRS is manifested by two or more of the following: temperature >38° C or <36°C, heart rate >90 beats per minute, respiratory rate >20 breaths per minute or $PaCO_2$ <32mmHg, and/or white blood cell count >12,000 cells/μL. Despite these criteria, however, a recent European Society of Intensive Care Medicine (ESICM/SCCM) survey revealed that 71% of physicians cite no common definition of sepsis.[2] This study demonstrated a perceived ambiguity in diagnosing sepsis and inspired the 2001 International Sepsis Definitions Conference, sponsored by SCCM, ESICM, ACCP, the American Thoracic Society, and the Surgical Infection Society.

The physicians who attended the 2001 conference determined that, while SIRS remains a useful concept, the criteria released in 1992 defining SIRS was "overly sensitive" and non-specific.[2] Investigators have since found certain biochemical features to be important in diagnosing sepsis. For example, elevated circulating levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), extracellular phospholipase A_2, and C-reactive protein have all been detected in patients meeting the 1992 SIRS criteria.[2] Doctors at the conference therefore hypothesized that immunologic and biochemical criteria would become the reference for

detecting sepsis in the future. Until studies support such a conclusion, however, an expanded list of signs and symptoms of sepsis may improve the diagnosis and treatment of the infection.

The Pathophysiology of Sepsis

Sepsis over-amplifies immune, inflammatory, and coagulation responses. Although infecting pathogens were traditionally associated with bacterial infection, research has shown that fungi and viruses also lead to sepsis. A 1998 study by Friedman and Vincent also indicated a shift from Gram-negative to Gram-positive bacteria in organisms causing sepsis.[3] The sequence of events triggered by Gram-negative bacteria in septic shock (acute circulatory failure plus severe infection) begins with lipopolysaccharide (LPB) binding to the LPB-binding protein in blood plasma, forming the LPS-LPB complex. This complex then binds to Toll-like Receptor-2 (TLR-2), a macrophage receptor, activating nuclear factor-κB (NF-κB), which then causes release of proinflammatory mediators such as TNF-α.[4] Proinflammatory mediator release attracts more macrophages, and the cycle repeats. Although such a sequence of events is known for Gram-negative bacteria, in the presence of Gram-positive bacteria it is less well defined.

Among the many cytokines involved in septic shock, TNF-α and interleukins (IL-1 and IL-6) have been studied most. TNF-α administration mimics typical symptoms of sepsis. For example, TNF-α administered to animals causes alterations in coagulation, pulmonary edema, and renal failure, while administration to human volunteers causes fever, hypotension, high cardiac output, and myocardial depression.[4] A study published in *Chest,* featuring 2,634 patients with severe sepsis who received either afelimamab (an anti-TNF antibody) or a placebo, shows that afelimamab leads to a 10% decrease in risk of mortality.[5] Furthermore, according to Pinski et al. and Casey et al., the degree of elevated TNF-α level is directly proportional to the severity of infection.[6,7] However, Hack et al. found that IL-6 correlates more closely with the severity of septic shock than TNF-α, suggesting that IL-6 levels may also pose as an indicator of the septic response.[8]

Management of Early Sepsis

Early sepsis can remain stagnant or progress to severe sepsis or septic shock. Movement along this spectrum involves circulatory abnormalities (intravascular volume depletion, peripheral vasodilation, myocardial depression, and increased metabolism), leading to an imbalance between oxygen delivery and demand, and resulting in tissue hypoxia or shock. Early recognition and treatment during transition toward septic shock is critical.[9] Increased knowledge of the inflammatory and immunosuppressive responses has enabled brisk diagnosis (within 6 hours) and treatment. Optimal management of sepsis involves early goal-directed therapy, lung protective ventilation, broad-spectrum antibiotics, and activated protein C.[10]

Early Goal-directed Therapy

Standard therapy based on physical findings, vital signs, central venous pressure, and urinary output fail to detect tissue hypoxia.[9] Early, goal-directed therapy, however, involves manipulating cardiac preload, afterload, and contractility to balance oxygen demand with delivery.[9] Rivers et al. conducted a controlled, randomized study involving 263 emergency room septic patients, 130 assigned to early goal-directed therapy and 133 to standard therapy.[9] In-hospital mortality was 30.5% in the early goal-directed therapy patients, compared with 46.5% in standard therapy.[9] Early goal-directed therapy patients received more fluids, transfusions, and dobutamine in the first 6 hours. During the interval from 7 to 72 hours, these patients had a mean central venous oxygen saturation of 70.4%, compared to 65.3% in standard therapy patients.[9] Early goal-directed therapy patients also had a lower lactate concentration (3.0 vs. 3.9 mmol/L), lower base deficit (2.0 vs. 5.1 mmol/L), and higher pH (7.4 vs. 7.36), than patients under standard care.[9]

During the period between 7 and 72 hours after hospital admission, mean Acute Physiology and Chronic Health Evaluation (APACHE II) scores were significantly lower in patients assigned to early goal-directed therapy compared to standard therapy (13.0 vs. 15.9).[9] The Simplified Acute Physiology Score (SAPS II) and Multiple Organ Dysfunction Score (MODS) were also lower in the early goal-directed therapy patients, indicating less severe organ dysfunction.[9] Early goal-directed therapy has

therefore been proven to enhance treatment and outcomes in severely septic patients.

Lung Protective Ventilation

Excessive tidal volume and repeated opening and closing of lung alveoli during mechanical ventilation cause acute lung injury which further complicates sepsis.[10] Lung-protective mechanical ventilation (tidal volume of 6 ml per kg of body weight compared with 12 ml per kg) has been shown to decrease mortality rate from 40% to 31%.[10] Although positive end-expiratory pressure (PEEP) decreases oxygen requirements, patients receiving standard PEEP levels suggested by the Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network have shown no significant decrease in mortality compared to patients treated with higher PEEP levels.[10] Furthermore, patients receiving mechanical ventilation need sedation to control the prolonged ventilation and risk of nosocomial pneumonia, although daily intermittent disruptions in sedation reduce risk of anesthesia.[10]

Broad Spectrum Antibiotics

The site of infection causing sepsis is usually unknown at the time of hospital admittance. Cultures are obtained and broad spectrum antibiotics administered until the specific pathogen is identified.[10] When deciding initial treatment, fungal prevalence, Gram-positive bacteria, resistant Gram-negative bacilli, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococcus*, and penicillin-resistant *pneumococcus* should all be considered in order to prevent inadequate antimicrobial therapy.[10,11] Harbarth et al. and Ibrahim et al. observed worse outcomes in patients with sepsis and septic shock–possessing pathogens unresponsive to the initial broad spectrum antibiotic treatments such as *Pseudomonas aeruginosa*.[11,12]

According to Ibrahim et al., antibiotic-resistant bacteria strains contribute to inadequate antimicrobial therapy associated with greater hospital mortality rates.[12] Between July 1997 and July 1999, Ibrahim et al. performed a prospective cohort study to determine the occurrence of bloodstream infections and the outcome of inadequate antimicrobial treatment. Of the 492 critically ill ICU patients evaluated, 147 received inadequate antimicrobial treatment for blood infections. The hospital

mortality rate of these patients (61.9%) was significantly greater than the mortality rate of patients receiving adequate therapy (28.4%).[12] Interestingly, *Candida* species were found to be the most abundant bloodstream infections among non-survivors, though infections most common in survivors were attributed to coagulase-negative *staphylococci* and oxacillin-sensitive *S. aureus*.[12] Efforts should therefore be aimed at reducing inadequate antimicrobial administration to critically ill patients with bloodstream infections attributed to resistant strains or *Candida*.

Activated Protein C

Administering 24µg per kilogram per minute of activated protein C (Drotrecogin Alfa) for 96 hours post goal-directed therapy, lung protective ventilation, and antibiotic treatment have proven to decrease mortality in severely septic patients.[10] Activated protein C has been approved for treating severe sepsis in patients with high risk of death as indicated by an APACHE II score of 25 or greater, or extreme organ dysfunction.[10] Such patients show a 13% decrease in mortality rate.[10] On the other hand, in patients with a low mortality risk, the Administration of Drotrecogin Alfa in Early Stage Severe Sepsis (ADDRESS) trial declares activated protein C ineffective.[10] According to the Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial, patients with an active hemorrhage, thromboocyopenia (platelet count less than 30,000 per mm^3), or a history of strokes, as well as patients receiving therapeutic anticoagulants, should be exempt from treatment with activated protein C treatment due to the high risk of bleeding.[10]

Evaluating and Controlling Sepsis

Once the specific pathogen has been determined in the patient, antibiotic treatment should be narrowed to decrease the risk of proliferating resistant strains. Management and support of affected organs, such as the kidneys, is also required in the critical care stage of sepsis.[10] Acute renal failure is common in severe sepsis and is correlated with an increased morbidity.[10] Recent studies, however, have shown difficulty in determining a beneficial treatment. For example, low dose dopamine (2 to 4µg per kilogram per minute) neither improves morbidity nor decreases the need for renal support, and sodium bicarbonate does not improve hemo-

dynamics or responses to vasopressor medications in septic patients with lactic acidosis.[10]

Hyperglycemia and Intensive Insulin Therapy

Hyperglycemia and insulin resistance are common in all sepsis cases.[10] Hyperglycemia acts as a pro-coagulant, induces apoptosis (cell death), prevents neutrophil function, increases infections, disrupts wound healing, and increases mortality.[10] Insulin defends against hyperglycemia by improving lipid levels while acting as an anti-inflammatory, anti-coagulant, and anti-apoptotic.[10] A study by Van den Berghe et al. involving administration of intensive insulin therapy to intubated surgical patients without sepsis suggests that intensive insulin therapy may be beneficial to patients with sepsis.[13] Intensive insulin therapy decreases the risk of death among patients remaining in the ICU for more than 3 days by decreasing the need for mechanical ventilation and renal replacement therapy, as well as minimizing peripheral neuromuscular dysfunction and bacteremia.[13]

Economic and Clinical Value of Rapid Diagnosis and Treatment

Roughly 750,000 Americans develop sepsis each year, with total costs averaging $22,100 per case and $16.7 billion annually.[14] Kost et al. calculated marginal penalties (reflecting unnecessary procedures, excess costs, and poor outcomes from inadequate treatment) based on clinical data from Kollef et al.[14,15] Value analysis revealed marginal penalties associated with increased ICU length of stay (LOS), catheterization, mechanical ventilation, tracheostomy, and higher mortality. Inadequate antimicrobial therapy in 655 ICU patients with blood infections caused an estimated $1,026 per patient expense from extended LOS.[14,15] According to Kost et al., these expenses represent "opportunity costs," or potential economic trade-offs, when considering new treatment advances. For example, nucleic acid testing is hypothesized to reduce marginal penalties by improving turnaround time (TAT) and enabling early sepsis diagnosis.[14] Rapid TAT, enabling appropriate antibiotic administration, has been demonstrated to improve mortality; therefore, timely diagnosis will improve economical and medical outcomes in septic patients.[14,16]

Nucleic acid testing is hypothesized to improve TAT and increase diagnostic efficiency during the critical time interval (TI_0) when patients

suffer the highest risk of dysfunctional sepsis cascades.[14] A study involving 66 septicemia patients hospitalized for 17 months showed that physicians alter antibiotic treatments over three time intervals: TI_1, when blood was collected to identify a positive culture; TI_2, when final MIC results were determined; and TI_3, roughly 72 hours after MIC results.[14] Antibiotic alterations during TI_1 are shown to increase mortality, while alterations tend to peak after positive blood culture results. Based on these alteration patterns, Kost et al. hypothesize that by identifying blood pathogens rapidly, or within 4 to 6 hours post admittance, nucleic acid testing will assist in accurate diagnosis and focused antibiotic therapy.[14] Such potential for enhanced survival and overall medical and economical improvement warrants clinical trials of nucleic acid PCR techniques.

Real-Time PCR Testing in Sepsis

During 2003, Klaschik et al. tested a prototype rapid real-time PCR system designed to detect bacterial DNA in less than 4 hours, including time from DNA preparation to final PCR results.[17] The DNA of 17 ICU-relevant bacteria species, such as *Pseudomonas aeruginosa, Escherichia, Streptococcus pyogenes, Staphylococcus epidermidis,* and *Staphylococcus aureus*, were extracted from water, plasma, and urine, then amplified by the "LightCycler" rapid real-time PCR instrument from Roche Diagnostics.[17] The DNA from all 17 bacteria were successfully extracted, detected, and classified under the correct Gram stain, while further species differentiation was determined by melting-curve analysis. Each rapid real-time PCR run determined the emitted fluorescence wavelength, 640nm for Gram-negative bacteria and 705nm for Gram-positive, and the melting temperature of both the hybridization probe and the PCR product.[17]

The results published in 2004 by Klaschik et al. show that all Gram-negative bacteria are distinguishable by differing melting points and fluorescence. However, this is not the case with all Gram-positive bacteria. *S. aureus* and *S. epidermidis* cannot be differentiated due to similar melting point characteristics. Therefore, these two organisms are both classified under "*Staphylococcus* species" until a specific internal probe is developed.[17]

Additional studies have shown blood culture–based antimicrobial treatments fail to achieve effectiveness in approximately 25% of patients with blood infections.[18] Inadequate anti-microbial treatments have also

contributed to marginal penalties and the increased risk of resistance, incomplete therapy, and re-hospitalization.[14,18,19] According to Peterson et al., molecular diagnostics have demonstrated reduced drug resistance development in microbial organisms.[20] Despite the qualitative species identification (i.e., Gram-positive or Gram-negative) and quantitative minimum inhibitory concentration provided by traditional culture methods, rapid pathogen detection techniques (such as real-time PCR) are needed to accelerate diagnosis and treatment of septicemia.

Current Research on Real-Time PCR Testing and Sepsis

Louie et al. are currently conducting a study involving 200 high-risk patients with line infections, cancer, neutropenic fever, AIDS, cellulites, pyelonephritis, GI infections, and so on. A 3 ml blood sample is drawn for PCR testing in parallel with samples obtained for blood culture from patients satisfying the SIRS criteria. The 3 ml of blood is subjected to bacteria and fungi nucleic acid testing with the Septifast LightCyler 2.0, which targets the internal transcribed spacer (ITS) in DNA. The PCR process involves 40 cycles of denaturation, annealing, and elongation. The optimal temperature for maximum probe binding is then determined (e.g. 51°C for *E. Coli*), and the PCR results are compared to the blood culture results and analyzed for clinical value.

Approximately 33% of septic patients are blood culture negative.[21] Louie et al. have also collected PCR results detecting pathogens unidentified by blood culture. For example, both PCR and urine culture identified *E. Coli,* but blood culture samples were negative after five days in a 91-year-old female treated for suspected aspiration pneumonia and urinary tract infection (UTI) upon hospital admittance.[21] Another patient was immediately prescribed Cefotaxime for a UTI when admitted into the hospital. Though the blood culture showed no growth, PCR results detected *S. aureus*. Two days later, after no sign of patient improvement, physicians "suspected" possible *S. aureus* infection and switched treatment to Nafcillin despite the remaining negative blood culture.[21] The patient quickly recovered. In this case, inadequate antimicrobial treatment could have been avoided with the rapid detection of *S. aureus* by PCR testing.

In addition to the work by Louie et al., Nam Tran's Ph.D. thesis at the University of California, Davis, will test the accuracy of real-time PCR in detecting *Candida glabrata.* According to Ben-Abraham et

al., *Candida glabrata* is associated with the highest mortality (73%) in patients with systemic infections.[18,20] Tran's study will consist of a bench component assessing the analytical sensitivity of the PCR assay, and a clinical component focusing on agreement between PCR vs. blood culture. During the bench study, human whole blood will be spiked with serial dilutions (10^{-1} to 10^{-9} in sterile saline) and tested on the LightCycler PCR instrument.[18] The dilutions used for PCR will also be tested on a blood agar plate to confirm the number of colony-forming units per milliliter (CFU/mL). Based on the SIRS criteria, the clinical study will involve 200 infection-prone patients with illnesses such as AIDS, cancer, organ transplant, end-stage renal disease, pulmonary disease, total parenteral nutrition, neutropenic fever, and so on.[18]

Samples for PCR testing will be collected at the same time as blood cultures, as in the experiment by Louie et al. Data collection will include blood culture results, relevant procedures supporting evidence of infection, demographics, patient mortality, LOS, antimicrobial therapy, and laboratory results such as CBC, WBC, and blood chemistry. Analysis will focus on the level of agreement between PCR and blood culture, with blood culture serving as the reference.[18] Tran hypothesizes real-time PCR will show equal or better performance characteristics compared to current blood culture methods when detecting organisms commonly found in patients with septicemia.[18] Tran et al. furthermore hope to identify detection limits such as the analytical sensitivity of the PCR assay in order to optimize sample collection, testing, and interpretation.[18] PCR assays may potentially improve TAT and antimicrobial therapy against pathogens such as *Candida sp*., while further reducing resistant strains. Ultimately, PCR testing may target specific patient populations who would benefit most from rapid pathogen detection—including immunosuppressed AIDS patients and post-surgical patients—thereby facilitating better outcomes through accelerated evidence-based and cost-effective treatment.[18]

References

1. Balk RA, Ely EW, Goyette RE. NISE *Sepsis Handbook*, 2nd Edition. Thomson Advanced Therapeutics Communications and Vanderbilt School of Medicine, 2004.

2. Levy, MM, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; 31:1250–6.

3. Friedman G, Vincent JL. Has the mortality of septic shock changed with time? *Crit Care Med* 1998; 26:2078–86.

4. Vincent, JL and Backer DD. Pathophysiology of septic shock. *Advances in Sepsis* 2001; 1:87–92.

5. Panacek EA, Marshall J, Fischkoff S, et al. Neutralization of TNF by a monoclonal antibody improves survival and reduces organ dysfunction in human sepsis: Results of the MONARCS trial. *Chest* 2000;118:88S (Abst).

6. Pinsky MR, Vincent JL, Deviere J. Serum cytokine levels in human septic shock: Relation to multiple-systems organ failure and mortality. *Chest* 1993; 103:565–75.

7. Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin levels correlate with survival in patients with sepsis syndrome. *Ann Intern Med* 1993;119:771–8.

8. Hack CE, De Groot ER, Felt-Bersma RJF, et al. Increased plasma levels of interleukin-6 in sepsis. *Blood* 1989; 74:1704–10.

9. Rivers, E, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001; 345:1368–77.

10. Russell, JA. Management of sepsis. *N Engl J Med* 2006; 355:1699–713.

11. Harbarth S, Garbino J, Pugin J, Romand JA, Lew D, Pittet D. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. *Am J Med* 2003; 115:529–35.

12. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. *Chest* 2000; 118:146–55.

13. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001; 345:1359–67.

14. Kost GJ, Tang Z, Tran NK, et al. Economic implications of optical diagnosis and treatment of sepsis—work in progress: Marginal penalties, antibotic alterations, and outcome hypotheses. *Scandinavian J Clinical Lab Investigations* 2003; 63 (S239):16–26.

15. Kollef MH, Sherman G, Ward S, Fraser VJ. Inadequate antimicrobial treatment of infections: A risk factor for hospital mortality among critically ill patients. *Chest* 1999; 115:462–74.

16. Tran NK, Kost GJ. Point of care testing in critical care medicine: Improving outcomes, cost effectiveness, and turnaround time. *Intl J Intensive Care* 2003.

17. Klaschik, S, et al. Detection and differentiation of invitro-spiked bacteria by real time PCR and melting curve analysis. *J Clinical Microbiol* 2004; 42:512–7.

18. Tran, NK. Multiplexed real-time PCR in sepsis: Determining analytical sensitivity and clinical value. Qualifying Exam Thesis Proposal. 2006. Pending publication.

19. Braun L, Riedal AA, Cooper LM. Severe sepsis in managed care: Analysis of incidence, one-year mortality, and associated costs of care. *J Manag Care Pharm* 2004; 10:521–30.

20. Ben-Abraham R, Keller N, Teodorovitch N, Barzilai A, Harel R, Brazilay Z, Paret G. Predictors of adverse outcome from candidal infection in tertiary care hospital. *J Infect* 2004; 49:317–23.

21. Louie RF, Tang Z, Tran NK, et al. Multiplexed simultaneous detection of bacteria and fungi by PCR-based nucleic acid testing for rapid diagnosis of multipathogen bloodstream infections and value mapping for evidence-based treatment of high risk critically ill patients. *American Association of Clinical Chemistry: 21st International Symposium.* "Refining Point of Care Testing Strategies for Critical and Emergency Care." Quebec, Canada, 29 Sept 2006.