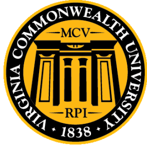

Virginia Commonwealth University
VCU Scholars Compass

Theses and Dissertations

Graduate School

2012

Use of Procalcitonin as a Biomarker of Bacterial Infection in Acute Liver Failure and Acute Liver Injury

Jody Balko
Virginia Commonwealth University

Follow this and additional works at: https://scholarscompass.vcu.edu/etd

Part of the Medicine and Health Sciences Commons

© The Author

Downloaded from

https://scholarscompass.vcu.edu/etd/2744

This Dissertation is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

المنارة للاستشارات

www.manaraa.com

© Jody A. Balko 2012
All Rights Reserved

المنارة للاستشارات

www.manaraa.com

Use of Procalcitonin as a Biomarker of Bacterial Infection in Acute Liver Failure and Acute Liver Injury

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

Jody Anne Balko
Bachelor of Science in Medical Technology, Georgia Southern College, 1979
Master of Medical Science in Clinical Chemistry, Emory University, 1992

Advisor: William J. Korzun, Ph.D.,
Associate Professor
Department of Clinical Laboratory Sciences

Virginia Commonwealth University
Richmond, Virginia
March 27, 2012

المنارة للاستشارات
www.manaraa.com

ACKNOWLEDGEMENTS

There are many people who have in one way or another guided me to this point. First, I would like to thank my committee members Dr. William Korzun, Dr. Teresa Nadder, Dr. Diane Dodd-McCue, Dr. Linda Hynan, and most especially Dr. William Lee. Thank you, Dr. Lee for giving me the opportunity to work with you on this project and many others over the years. I would also like to give a special thank you to Dr. Frank H. Wians, Jr. for giving me the opportunity explore my limits and helping me believe that I could accomplish this journey.

I especially want to remember my grandparents Ollie Mae and Owen Davis. They were not here to see this journey but without their love and support, it might never have begun. They never understood my love of science but encouraged me to keep going as far as I could. I dedicate this to their memory. Also my father, Bernie Crouse, he started this journey with me, but missed the end by such a short time.

This adventure would not have been nearly as much "fun" without Cohort 2008 to share it with. Emily, Kevin, Maribeth, Kate, Jason, Scott, Kristie, Laurie, Ferell, and Andi – we have shared many of life's ups and downs together, as well as classes and comps. There couldn't have been a better group to travel with.

المنارة للاستشارات

www.manaraa.com

My thanks for the support of my co-workers – Corron, Nahid, Rehana, Theresa, Stacey, Sycil, and Paola. Thanks for covering, for putting up with the worry over missed timelines, and for all of the help you gave me to see this project completed.

I would also like to thank Jim Freeman and the others at Siemens Healthcare in Tarrytown, NY. Their help and support made the completion of this project much easier and meant that it was accomplished in as short a time period as possible.

Finally, Wayne Rule, your support through the good times and the bad, made it possible to finish this journey. I will never be able to thank you enough. Thank You!

المنارة للاستشارات
www.manaraa.com

TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES ix

GLOSSARY OF ABBREVIATIONS AND DEFINITIONS OF TERMS. x

ABSTRACT xiii

CHAPTER 1: INTRODUCTION 1
Purpose of This Study. 3
Specific Aims 4
Summary. 5

CHAPTER 2: LITERATURE REVIEW. 6
Liver Structure and Physiology. 6
Acute Liver Failure and Acute Liver Injury 9
Bacterial Infection and Sepsis. 14
SIRS, Sepsis, Severe Sepsis, and Septic Shock 15
Methods to Identify Bacterial Organisms. 20
Procalcitonin 23
Procalcitonin Testing 24
PCT as a Biomarker of Bacterial Infection or Sepsis. .28
PCT Use to Guide Antibiotic Therapy 36
Procalcitonin and Liver Disease. 41
Summary. 45
Significance of This Study 46

CHAPTER 3: METHODS. 47
Research Design. 47
Specific Aims, Hypotheses, and Study. 48

www.manaraa.com

Databases. 50
Acute Liver Failure Database. 51
Acute Liver Injury Database. 52
Liver Disease Database. 53
Nucleic Acid, Serum, and Tissue Repository for the Study of Liver Diseases Database. 55
Sampling. 55
Specific Aim One. 55
Specific Aim Two. 61
Specific Aim Three. 62
Testing. 63
Specimen Procurement and Retrieval. 63
ADVIA Centaur Immunoassay Analyzer Testing. 63
Statistical Analysis 67

CHAPTER FOUR: RESULTS. 70
Assay Statistics. 70
Specific Aim One. 71
Sample Selection. 72
Demographic Information. 74
Statistical Results. 76
Specific Aim Two. 86
Sample Selection. 86
Demographic Information. 86
Statistical Results. 87
Specific Aim Three. 95
Sample Selection. 95
Demographic Information. 97
Statistical Results. 97
Summary. 102

CHAPTER FIVE: DISCUSSION. 104
Overview of the Problem. 104
Discussion of the Study. 105
Specific Aim One. 106
Specific Aim Two. 112
Specific Aim Three 115
Conclusions. 118

المنارة للاستشارات

www.manaraa.com

Limitations of the Study. 119
Recommendations for Future Studies. 125

REFERENCES 127

APPENDIX A. 140

VITA. 144

المنارة للاستشارات
www.manaraa.com

LIST OF TABLES

Table 1: KRYPTOR vs. ADVIA Centaur Assays for SPCTC 29

Table 2: Published Studies of PCT Guided Antibiotic Therapy vs. SOC. 39

Table 3: Comparison of SPCTC Values in Appropriate and Inappropriate Antibiotic Therapy. 40

Table 4: SPCTC and Child-Pugh Scores in Patients with Cirrhosis, With and Without Infection. 43

Table 5: SPCTC in Patients with Liver Cancer. 44

Table 6: Databases. 51

Table 7: Severe Sepsis Signs and Symptoms. 58

Table 8: Categories and Numbers of Subjects Desired for Specific Aim One 61

Table 9: PCT Assay Imprecision. 71

Table 10: Category and Sample Availability of ALF and ALI Subjects. 72

Table 11: Number of Subjects Tested for PCT Based Upon Severity Category and ALF Etiology. 73

Table 12: Demographic Information for Patients Based Upon Severity of Illness Classifications 75

Table 13: Biodata and Laboratory Values for Patients Based Upon Severity of Illness Classifications 76

Table 14: Comparison of tSPCTC in Non-Infected Patients to the Published Reference Value (<0.1 ng/mL) 79

www.manaraa.com

Table 15: Results for post hoc Pairwise Testing Using the Dunn Method 79

Table 16: Demographic and Biodata Results Based Upon the Calculated Infection Cut-off Value of 1.62 ng/mL 83

Table 17: Comparison of Median SPCTC Results of Subjects in Specific Aim One Sorted by Category and Etiologies 84

Table 18: Demographic Information for 34 Patients Analyzed for SPCTC to Detect Bacterial Infection 88

Table 19: Demographic Data for All Subjects and Each of the Three Subgroups on the Day of Infection 93

Table 20: Friedman Test for PCT Results for All Samples and Subsets A, B, and C. 94

Table 21: Number of Samples Tested by Days and Outcomes. 96

Table 22: Demographic Information Based on Outcomes for Patients with a Documented Infection on Day of Admission to the ALF or ALI Studies. . . .98

Table 23: Laboratory Values for Patients with a Documented Infection on the Day of Admission to the ALF or ALI Studies 99

Table 24: Change in Medians of SPCTC From Day 1 in TFS and DoT Subjects. 101

Table 25: Summary of Study Hypotheses 106

Table 26: Comparison of SIRS Category SPCTC Results by SIRS Category Between This Study and Previously Published Results. 112

المنارة للاستشارات

www.manaraa.com

LIST OF FIGURES

Figure 1: Schematic of Procalcitonin Molecule 23

Figure 2: Siemens PCT Reagent Components 64

Figure 3: Sample + Ancillary Reagent 65

Figure 4: Add Solid Phase and Lite Reagent 66

Figure 5: SPCTC by SIRS Classifications 77

Figure 6: Receiver Operator Curve for the Detection of Infection in ALF and ALI Patients Using SPCT. 81

Figure 7: Median SPCTCs by SIRS Classifications Sorted by Etiologies. 84

Figure 8: Comparison of Kruskal-Wallis Mean Ranks for All, APAP, and Other Etiologies 85

Figure 9: SPCTC Results of All Subjects for Three Days Prior to and the Day of Infection 89

Figure 10: SPCTC Values for DoI and Three Days Prior to DoI 89

Figure 11: SPCTC Results for Subjects in Subgroup A 90

Figure 12: SPCTC Results for Subjects in Subgroup B. 91

Figure 13: SPCTC Results for Subjects in Subgroup C. 92

Figure 14: Median tSPCTC per Day Sorted by Outcome. 99

Figure 15: Estimates of Visit Day * Outcome by Day. 102

للاستشارات المنارة

www.manaraa.com

GLOSSARY OF ABBREVIATIONS AND DEFINITIONS OF TERMS

^{o} C	Degrees Celsius
ACCP	American College of Chest Physicians
AE	Acridinium ester
AFLP	Acute fatty liver of pregnancy
AIH	Autoimmune hepatitis
ALF	Acute liver failure
ALI	Acute liver injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APAP	acetaminophen or paracetamol
aPTT	Activated partial thromboplastin time
ARDS	Adult respiratory distress syndrome
AST	Aspartate aminotransferase
ATS	American Thoracic Society
BBB	Blood brain barrier
CCLD	Clinical Center for Liver Disease
CenPCT	ADVIA Centaur BRAHMS PCT
CGRP	Calcitonin gene-related peptide
CRF	Case report form
CV	Coefficient of variation
D-1	One day prior to the day a positive culture was collected
D-2	Two days prior to the day a positive culture was collected
D-3	Three days prior to the day a positive culture was collected
df	Degrees of freedom
DNA	Deoxyribonucleic acid
DoI	Day of infection (i.e., day a culture reported as positive was collected)
DoT	Death or Liver Transplant
ED	Emergency department
ESICM	European Society of Intensive Care Medicine
EU	European Union
FDA	Food and Drug Administration
FiO2	Fraction of inspired oxygen
FISH	Fluorescence in situ hybridization
GGT	Gamma-glutamyltransferase
HE	Hepatic encephalopathy

www.manaraa.com

HLA	Human leukocyte antigen
ICU	Intensive care unit
ILMA	Immunoluminometric assay
Inf	Infected or Infection
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous
LDD	Liver Disease Database
LRTI	Lower respiratory tract infection
MAP	Mean arterial pressure
MTC	Medullary thyroid cancer
NI	Non-infected or No Infection
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NSTLD	Nucleic Acid, Serum, and Tissue Repository of the Study of Liver Diseases
PaO2	Partial pressure of oxygen in arterial blood
pCO2	partial Carbon dioxide level in arterial blood
PCR	Polymerase chain reaction
PCT	Procalcitonin
PMP	Paramagnetic particles
ProHOSP	Procalcitonin Guided Antibiotic Therapy and Hospitalization in Patients with Lower Respiratory Tract Infections Study
PRORATA	Procalcitonin to Reduce Antibiotic Treatment in Acutely ill patients Trial
PT	Prothrombin time
r	Correlation coefficient
RLU	Relative light units
ROC	Receiver operator curve
SAF	Sterile ascitic fluid
SBP	Spontaneous bacterial peritonitis
SCCM	Society of Critical Care Medicine
SD	Standard deviation
SIRS	Systemic Inflammatory Response Syndrome
SIS	Surgical Infection Society
SOC	Standard of care
SOFA	Sequential Organ Failure Assessment
SPCTC	Serum procalcitonin concentration
SVO2	Mixed venous oxygen saturation
$t_{1/2}$	Half-life
TFS	Transplant free survival
TRACE	Time Resolved Amplified Cryptate Emission
tSPCTC	Transformed serum procalcitonin concentration (tSPCTC = Log10 (SPCTC + 1))

المنارة للاستشارات
www.manaraa.com

UNOS	United Network for Organ Sharing
US	United States
UTSW	University of Texas Southwestern Medical Center at Dallas
VAP	Ventilator-associated pneumonia
WBC	White blood cell

المنارة للاستشارات
www.manaraa.com

ABSTRACT

USE OF PROCALCITONIN AS A BIOMARKER OF BACTERIAL INFECTION IN ACUTE LIVER FAILURE AND ACUTE LIVER INJURY

By Jody Anne Balko, Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2012

Advisor: William J. Korzun, Ph.D., Associate Professor, Department of Clinical Laboratory Sciences

Infections in patients with acute liver failure (ALF) and acute liver injury (ALI) are a frequent occurrence. Because ALF and ALI patients share many of the same clinical features as patients with severe sepsis and septic shock, identifying an infection based upon clinical manifestations is extremely difficult. Bacterial culture and sensitivity reports require 24 to 72 hours to be finalized after the need for a culture is suspected and obtained. During this time period, ALF and ALI patients are either not receiving required antibiotic therapy, receiving antibiotic therapy that is not required or not appropriate for the infecting bacterial pathogen, or receiving the correct antibiotic prophylaxis. Receiving an antibiotic that is not needed or inappropriate adds another level of complexity to the ALF and ALI patients because antibiotics may exacerbate liver dysfunction.

المنارة للاستشارات

www.manaraa.com

The purpose of this study was to determine the utility of serum procalcitonin concentrations (SPCTC) as a biomarker of bacterial infections in patients with acute liver failure (ALF) and acute liver injury (ALI). This three part study measured SPCTC retrospectively on samples from ALF and ALI patients who were prospectively enrolled in the United States Acute Liver Failure Study Group (USALFSG) ALF and ALI studies. In the first part of the study, subjects were categorized according to how many SIRS continuum components they had and whether there was a documented infection. In the second part, serial samples on subjects who developed infections were identified. And, in the third part, serial samples on subjects diagnosed with infection on day one of the study and categorized based upon transplant free survival (TFS) or death and/or liver transplant (DoT) were identified.

Procalcitonin was not found to be useful in identifying infection in the ALF and ALI patient populations. A cut-off for indication of infection was calculated to be 1.62 ng/mL using receiver operator curve (ROC) analysis. Despite the fact that there was an overall increase in SPCTC as the severity of illness increased in patients with a documented infection, there were confounding variables including antibiotic use, missing data, and small sample size that may have contributed to the poor sensitivity and specificity (0.643 and 0.620 respectively) calculated as part of the ROC analysis.

SPCTC values appeared to be increased in subject with acetaminophen (APAP) toxicity and may have affected the cut-off, sensitivity, and specificity results. Increased SPCTC values were seen in APAP subjects who did not have a documented infection. It

www.manaraa.com

is unknown at this time if the SPCTC were increase due to liver damage, an undiagnosed infection, or as a result of increase cytokine production due to the APAP toxicity.

Serial PCT concentrations in patients who achieved TFS showed a greater decrease over time than those of patients who died or received a liver transplant, however, the TFS group contained a large portion of APAP subjects. Further prospective studies are needed to determine the extent of interference with SPCTC in patients with APAP toxicity and to better define the PCT concentration cut-off between infection and no infection in the ALF and ALI populations.

المنارة للاستشارات

www.manaraa.com

CHAPTER 1: INTRODUCTION

Infections are a frequent occurrence in patients diagnosed with acute liver failure (ALF) and acute liver injury (ALI). Overall mortality in patients with ALF has been estimated at 30 – 40%. Approximately 44% of ALF patients are listed for liver transplantation with 12 – 25% of all ALF patients receiving a liver transplant (Bower, Johns, Margolis, Williams, & Bell, 2007; Lee, Squires, Nyberg, Doo, & Hoofnagle, 2008). Bacterial infections are reported to occur in 40% - 90% of ALF patients (Rolando et al., 2000; Vaquero et al., 2003). The mortality rate due to bacterial infection (or suspected bacterial infection) in the general population increases from approximately 10% in patients with systemic inflammatory response syndrome (SIRS), to approximately 50% in patients with septic shock (Brun-Buisson, 2000; Rangel-Frausto et al., 1995). ALF has many of the same characteristics of septic shock and the presence of SIRS in ALF is associated with a poor prognosis (Antoniades, Berry, Wendon, & Vergani, 2008). Studies have shown a link between bacterial infection and increasing mortality in ALF (Rolando et al., 1990; Rolando et al., 2000). Another study has indicated a relationship between bacterial infection and the progression to stage III-IV hepatic encephalopathy (HE) and a decrease in spontaneous (survival without liver transplant) survival (Vaquero et al., 2003). However, there are no studies specifically looking at bacterial infection in the ALF population related to the SIRS spectrum and to clinical outcome. Currently,

المنارة للاستشارات
www.manaraa.com

methods to detect and identify bacterial infections and antibiotic sensitivities require at least 24 to 72 hours to produce results. Furthermore, cultures may be negative even if an infection is present (Fenollar & Raoult, 2007). Various methods utilizing molecular techniques have been developed in an effort to speed up and/or improve identification of infection-causing bacteria. While these techniques may improve the time to result for organisms that require longer incubation periods (days to weeks) to grow and isolate, the techniques do not improve the time to result for pathogens requiring shorter incubation periods (24 – 48 hours) to grow and isolate (Sabet, Subramaniam, Navaratnam, & Sekaran, 2006; Stefani, 2009).

A biomarker that can provide an immediate determination that a bacterial infection is present and can give an indication of severity would provide a major improvement in the ability to treat ALF and ALI patients who have a bacterial infection. Procalcitonin (PCT) is a biomarker of inflammatory response that requires one to two hours to obtain a result and tends to increase with the severity of the infection. Studies indicate that elevated PCT values are associated with most types of bacterial infections (Brunkhorst, Wegscheider, Forycki, & Brunkhorst, 2000; P. E. Charles et al., 2008; Christ-Crain, M., Jaccard-Stolz, D., Bingisser, R., Gencay, M. M., Huber, P. R., Tamm, M., et al. (2004). PCT has a half-life of 25 to 30 hours suggesting that a short-term rise or fall in the serum PCT concentration (SPCTC) could be indicative of a change in a patient’s infection status. The PCT molecule is stable in serum or plasma samples for up to 24 hours at 4°C, making accurate measurement of PCT feasible. SPCTC is a potential

المنارة للاستشارات
www.manaraa.com

candidate for measurement in the clinical laboratory to monitor patients for bacterial infection and sepsis (Jin & Khan, 2010).

In studies in patients with sepsis, pneumonia, and other types of infections, PCT has been shown to be a good indicator of bacterial infection and a useful tool in guiding antimicrobial therapy (Muller, Christ-Crain, & Schuetz, 2007). In patients with liver disease, PCT has been examined in patients who have had liver transplant and in patients hospitalized with chronic liver disease (cirrhosis), but there are no studies of patients with ALF or ALI (Elefsiniotis et al., 2006).

Purpose of This Study

The purpose of the study was to retrospectively examine the levels of PCT in patients with ALF and ALI with and without evidence of bacterial infection to determine the usefulness of procalcitonin as a biomarker of infection in ALF and ALI patients. The U.S. Acute Liver Failure Study Group (USALFSG) maintains ALF and ALI databases to prospectively collect clinical information about ALF and ALI that is used to conduct clinical trials aimed at improving treatment for ALF and ALI patients. The USALFSG was established in 1998 with pilot funding from the FDA and is currently supported by the National Institute of Health (NIH).

The ALF/ALI databases include 632 documented cases of infection (248 blood, 304 urine, 49 catheter, 21 wound, 17 ascites, 238 tracheal aspirates, and 48 others (source not specified)) from a total of 1829 patients. This study examined the utility of SPCTC as an indicator of bacterial infection in ALF/ALI patients.

المنارة للاستشارات
www.manaraa.com

Specific Aims

There were three main aims for this study:

Specific Aim 1: To determine if there are differences in SPCTCs between non-infected ALF and ALI patients and non-infected chronic liver disease patients, between infected and non-infected ALF and ALI patients, and between ALF and ALI patients with different severities of infection and those published for the general population.

This was determined by measuring SPCTCs from sera that were collected from ALF and ALI patients who were prospectively enrolled in the ALF and ALI studies with a documented history of infection, ALF and ALI patients without an infection, and chronic liver disease patients without an infection. Chronic liver disease subjects were prospectively enrolled in one of two investigator-initiated databases maintained at the University of Texas Southwestern Medical Center (UTSW): The Liver Disease Database and the Nucleic Acid, Serum, and Tissue Repository for the Study of Liver Diseases (NSTLD). The results were analyzed according to etiology of liver disease, the presence or absence of infection, and the severity infection based upon clinical signs and symptoms.

Specific Aim 2: Determine the utility of SPCTC as a screening biomarker for infection in ALF and ALI patients

SPCTC was measured in sera collected on the three days prior to diagnosis of infection and on the day an infection was identified (i.e., the day a positive culture was obtained),

المنارة للاستشارات
www.manaraa.com

to determine whether PCT will allow an earlier indication of infection than clinical symptoms or culture findings.

Specific Aim 3: Determine if SPCTC in ALF and ALI patients with bacterial infection on Day 1 of the ALF or ALI study is associated with patient outcomes.

SPCTCs were measured in serial samples from ALF and ALI patients with known infections, to determine whether there are differences in the SPCTCs between patients with unfavorable outcomes (death or transplant) and those with favorable outcomes (transplant free survival).

Summary

Infection in ALF and ALI patients is a serious complication that may be difficult to detect because ALF and ALI subjects have clinical manifestations that are similar to septic shock. In addition, reports of bacterial cultures require 24 – 72 hours before they are available to the clinician.

SPCTC has been proposed as a biomarker of infection/sepsis that can reduce the time needed to detect bacterial infections and thereby allow earlier initiation of antimicrobial therapy. Multiple studies show that SPCTC has good sensitivity for detection of bacterial infection and indicate that SPCTC can decrease the length of antibiotic treatment. While there are multiple studies relating SPCTC to the detection of infection, there have only been a few studies on patients with chronic liver disease and none on ALF or ALI patient populations. In this study, the efficiency of SPCTC to detect infection and to predict outcomes in ALF and ALI patients was investigated.

المنارة للاستشارات
www.manaraa.com

CHAPTER 2: LITERATURE REVIEW

The following chapter will present background information relevant to procalcitonin, acute liver failure and injury, the pathophysiology of infection and sepsis, and the need to identify infections quickly. First, a description of procalcitonin, its origin, and its use as a biomarker for detecting and monitoring treatment of infection and sepsis will be presented. Next, information describing acute liver failure and acute liver injury and the need for a rapid determination of infection or sepsis in these populations will be presented. Next, information describing the pathophysiology of infection and sepsis will be presented. An overview of current methods for the diagnosis of bacterial infection and the strengths and weaknesses of these methods will be presented. Finally, background relating to the rationale for using procalcitonin to detect and monitor treatment of infection and sepsis in patients with acute liver failure or injury will be discussed.

Liver Structure and Physiology

The liver is composed of single cell thick sheets of hepatocytes arrayed around the central vein forming a hexagon. The hepatocyte sheets are separated by sinusoidal spaces that contain the Kupffer cells, endothelial cells, and stellate cells. The sinusoidal spaces allow blood flow from the hepatic artery and portal vein to the central vein. The canaliculi between the hepatocytes drain into ductules that empty into the bile duct. The

www.manaraa.com

hepatic artery, portal vein, and bile duct are located in the portal tracts located in the corners of the hexagons (Khalili, Liao, & Nquyen, 2010).

Hepatocytes compose approximately 80% of the total number of liver cells in an adult liver. Hepatocytes are the “metabolic factories” of the liver and are involved in carbohydrate, fat, and protein metabolism as well as the production of bile. An important feature of the hepatocyte is its ability to regenerate if a portion of the liver is lost or removed (Barrett, 2006).

Kupffer cells, which develop from the macrophage lineage, line the sinusoidal endothelium on the blood stream side of these cells and have highly active phagocytic properties, scavenging colonic bacteria that enter the bloodstream from the intestines by way of the portal vein. Activation of Kupffer cells by host or foreign antibodies may result in the production of cytokines and other inflammatory mediators that contribute to liver injury (Barrett, 2006).

Endothelial cells lining the hepatic sinusoids have a unique structure that allows macromolecules (such as albumin bound with various substances) produced by the hepatocytes, to move out of the cells into the blood stream while preventing formed elements in the blood (e.g., red blood cells, white blood cells, and platelets) from entering the hepatocytes. The structure also allows remnant macromolecules back into the hepatocyte. The structure of the sinusoidal endothelial cells is contractile and is controlled in part by various hormones and neurotransmitters. If the sinusoidal endothelial cells are damaged, passage of these macromolecules into the hepatocyte can be disrupted (Barrett, 2006).

المنارة للاستشارات
www.manaraa.com

Stellate cells store a variety of lipids, the most predominant of which is vitamin A. Stellate cells play a major role in liver injury by responding to inflammatory cytokines. When activated, the stellate cells lose their stores of vitamin A and up-regulate the production of extra-cellular matrix materials, such as collagen, which is deposited in the space between the hepatocytes and the sinusoidal endothelial cells, causing a disruption of hepatic function and leading to cirrhosis (Barrett, 2006).

Liver disease (acute or chronic) may result from impairment or death of one or any combination of the liver cell types. Liver function tests are the most commonly used method to assess liver injury. Liver function tests include measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), albumin, total and direct bilirubin, and plasma prothrombin time (PT) [converted to international normalized ratio (INR)] and ammonia. Liver function tests may be abnormal due to death of the hepatocytes (AST, ALT), failure of the liver to secrete bile called cholestasis (bilirubin, GGT, ALP), or disruption of metabolic and synthetic functions (PT/INR, ammonia, albumin). The degree to which the individual liver function tests are affected depends upon which cellular structures or functions are damaged and the amount of damage that occurs (Khalili et al., 2010; Merriman & Peters, 2003; Moseley, 2003; Barrett, 2006).

As the degree of damage to the liver increases and ammonia levels rise, hepatic encephalopathy (HE) may occur. While the pathophysiology of HE is not completely understood, what is known is that the disruption of the urea cycle in the liver leads to an increase in ammonia and other toxins. In ALF, massive astrocyte swelling and cerebral

المنارة للاستشارات
www.manaraa.com

edema due to an increase in glutamine production characterize HE and increased vasodilation possibly due to systemic release of gut-derived endotoxins, pro-inflammatory cytokines, and stimulation of nitric oxide synthase. As blood ammonia levels increase, cerebral ammonia levels also rise due to an impaired blood brain barrier (BBB) as the result of vasodilation. Increasing cerebral ammonia levels lead to the increased production of glutamine from glutamate by glutamine synthetase in the astrocyte, which is the only cell in the brain that contains this enzyme. Increased glutamine production results in astrocyte swelling. As the astrocytes swell, more ammonia is allowed to cross the BBB increasing the glutamine levels further and eventually resulting in cerebral edema and ultimately death by cerebral herniation (Barrett, 2006; Mendler, Donovan, & Blei, 2003).

Acute Liver Failure and Acute Liver Injury

Acute liver failure is characterized by severe liver cell dysfunction that occurs suddenly in patients with no known underlying liver disease. It is also known as fulminant hepatitis or fulminant hepatic failure. Although ALF was first described as a specific disease in the early 1950s, it was not until 1970 that it was defined as fulminant hepatic failure including HE. HE is cerebral dysfunction without structural abnormalities in the presence of liver disease. The extent of the dysfunction is graded (West Haven criteria grades) I – IV where grade I represents minimal dysfunction (trivial lack of awareness or shortened attention span) and grade IV represents coma (unresponsive to stimuli) (Ferenci et al., 2002; Trey & Davidson, 1970). The definition of ALF has been revised to include certain chronic liver diseases that can present as ALF but in retrospect,

المنارة للاستشارات

www.manaraa.com

have been present but unrecognized, specifically Wilson's Disease and hepatitis B. These two diseases are unique in having very rapid onset of acute liver injury in the presence of low grade chronic form of the disease (Khashab, Tector, & Kwo, 2007; Lee, 2003; Trey & Davidson, 1970).

In the United States (US), ALF is estimated to affect between 2000 and 2800 people per year (Bower et al., 2007; Khashab et al., 2007; Ostapowicz et al., 2002; Schiodt et al., 1999). Patients admitted to the hospital with ALF typically demonstrate increased levels of liver enzymes in serum, the abnormal coagulation (coagulopathy indicated by prothrombin time (PT) greater than 15 seconds or international normalized ratio (INR) greater than 1.5), plus hepatic encephalopathy and without evidence of cirrhosis (Lee & Seremba, 2008; Ostapowicz et al., 2002; Schiodt et al., 1999).

There are multiple etiologies of ALF. Previously, viral hepatitis (hepatitis A, B, and C) was the most common cause of ALF in the US (Schiodt et al., 1999). Currently, acetaminophen overdose constitutes the largest single cause of ALF accounting for 46% of all cases. Other etiologies include drugs (11%), hepatitis B virus (7%), hepatitis A virus (3%), autoimmune hepatitis (5%), ischemia (4%), and Wilson's disease (2%). Diseases or events, other than those listed previously, cause 7% of ALF cases and 14% of cases are from indeterminate causes (Lee & Seremba, 2008).

The etiology of ALF varies worldwide. Western countries (including Australia) document the majority of their ALF cases as being due to acetaminophen (paracetamol) toxicity. The United Kingdom reports a high prevalence of suicidal overdoses using

المنارة للاستشارات
www.manaraa.com

acetaminophen in the 1990s, accounting for 73% of ALF cases (Ostapowicz et al., 2002). That has decreased in part due to legislation to restrict sales of acetaminophen to a level of 57% (W. Bernal, Auzinger, Dhawan, & Wendon, 2010). In Asia and other developing countries, viral hepatitis (hepatitis A, B, and E) remains the leading cause of ALF. There is no vaccine for hepatitis E and vaccines for hepatitis A and B are less available in these countries. Hepatitis A and E are mainly transmitted by fecal-oral transmission routes and are closely associated with poor hygiene and sanitation. Hepatitis E is now the most common cause of ALF in India, Pakistan, China, and Southeast Asia (W. Bernal et al., 2010). The unavailability of many of the therapeutic drugs known to cause ALF in these countries is another reason why viral hepatitis is the leading cause of ALF in developing countries (W. Bernal & Wendon, 2000; Boeker, 2001; Ostapowicz et al., 2002; Polson & Lee, 2007).

Common therapeutic (both prescription and non-prescription) drugs that have been documented as causing ALF include acetaminophen (as previously noted), anti-tuberculosis, antiepileptic, and antibiotic pharmaceuticals. Antibiotics that have frequently been cited as causing ALF include isoniazide, sulfa-related compounds, quinolones, amoxicillin, and flucloxacillin. Telithromycin (Ketek), a recently released antibiotic, underwent a subsequent review by the US Food and Drug Administration (FDA) advisory panel, which resulted in severely limited future use and a "black box warning". A "black box warning" is a warning mandated by the FDA to appear on the full prescribing information of a prescription medication. Such a warning must be printed inside a box with a black outline at the top of the information sheet used by doctors and

المنارة للاستشارات
www.manaraa.com

pharmacists. A modified version of the same warning will appear on the patient information sheet. The restrictions resulted from numerous reports of ALF with short onset (as few as two days from initial use to symptoms of ALF), presence of abdominal pain, fever, and ascites (Lee & Seremba, 2008; Mindikoglu, Magder, & Regev, 2009).

While viral hepatitis and drug induced liver injury account for the majority of ALF worldwide, other etiologies are known. Autoimmune hepatitis (AIH) is an immune-mediated disease in which human leukocyte antigen (HLA) class II antigens are present on the surface of the hepatocyte and are the apparent target of the immune attack by the body's immune system. The immune response is complex and may involve T-cell activation, adhesion between immune synapses, cytokine production, and T-cell receptor configuration (Heathcote, 2003). Ischemia can result from a reduction of the blood flow to the liver due to systemic circulatory collapse or occlusion of the hepatic vein. Systemic circulatory collapse may result from loss of intravascular volume (hypovolemia) due to blood loss or other fluid loss due to massive diarrhea, congestive heart failure, or septic shock. Occlusion of the hepatic artery (Budd-Chiari Syndrome) may result from thrombus, fibrous obliteration, or tumor invasion (DeLeve, 2003). Metabolic anomalies result in the disruption of pathways that are important in the synthesis or transport of proteins in the liver, of which Wilson's disease (progressive accumulation of copper in hepatocytes) is the most common form seen in the US. Acute liver disease during pregnancy may also result in acute liver failure (Sokol, 2003). While there are three main forms of acute liver disease in pregnancy, acute fatty liver of pregnancy (AFLP) is the form most commonly seen in ALF. The cause of AFLP is unknown. Mild symptoms of

المنارة للاستشارات
www.manaraa.com

liver disease generally begin near 35 weeks gestation and may quickly progress to ALF and death (of mother and baby) if delivery is not initiated (Zimmermann & Christman, 2003).

Spontaneous recovery from ALF occurs in less than 50% of patients. Approximately 40% are placed on liver transplant waiting lists, but only 25% of the overall ALF population, receive a liver graft. Overall, 30% of patients diagnosed with ALF die within 21 days of the onset of illness (Bower et al., 2007; Lee & Seremba, 2008). Causes of death include cerebral edema, multi-organ failure, sepsis, cardiac arrhythmia or arrest, and respiratory failure (Ostapowicz et al., 2002).

Acute liver injury (ALI) is similar to ALF in that it is characterized by sudden severe liver cell dysfunction in patients with acute illness of less than 26 weeks duration (less than 2 weeks for acetaminophen etiology) but with no evidence of HE at the time of hospital admission. HE is diagnosed by neuropsychiatric evaluations focusing on mental and motor status (Ferenci et al., 2002). If HE develops, the patient's diagnosis changes to ALF. At the time of hospital admission, patients with ALI, as defined by the USALFSG, present with increased liver enzymes, and coagulopathy (prothrombin time (PT) greater than 20 seconds or international normalized ratio (INR) greater than 2.0) but do not demonstrate any encephalopathy.

When ALF is described in gastroenterology textbooks (Mendler et al., 2003), there is no distinction between ALF and ALI, as HE is graded as 0, 1, 2, 3, or 4. All information describing ALF and patient demographics above are based upon the ALF definition that does not include the ALI patients who have a HE grade of 0. Information

www.manaraa.com

characterizing the ALI subject population will be presented below, however, for the purposes of this study ALF and ALI will be distinguished only by their coma grade: ALI patients have a presenting coma grade of zero and ALF patients and ALI patients who develop HE have coma grades of 1 – 4. Throughout the rest of this discussion, unless a distinction between ALF and ALI is necessary, ALF will be used to refer to both ALF and ALI subjects in this study.

The etiologies of ALI are the same as those of ALF. While there are no figures available that document the number of ALI cases in the US, the USALFSG has estimates of the number of the various etiologies and the outcomes of ALI. Of the 163 ALI cases in the USALFSG database collected prior to October 1, 2010, 23% went on to develop HE and were transferred to the ALF database. Ninety one percent of the ALI cases went on to attain transplant free survival while 7% required a liver transplant and 2% died. As with ALF, acetaminophen poisoning accounts for the largest percentage of ALI cases (60%). Autoimmune hepatitis (10%) and drug-induced liver injury (9%) account for the next two largest numbers of cases. Other etiologies include hepatitis B virus (5%), hepatitis A virus (1%), mushroom poisoning (2%), shock (<1%), hepatitis E virus (<1%), and acute fatty liver of pregnancy (<1%). Diseases or events other than those listed caused 2.5% of ALI cases and cases from indeterminate causes account for 8% of the cases (United States Acute Liver Failure Study Group, 2011).

Bacterial Infection and Sepsis

Infection is a pathologic process that is initiated when normally sterile tissue or fluid or a body cavity is invaded by pathogenic or potentially pathogenic microorganisms

المنارة للاستشارات
www.manaraa.com

(Levy et al., 2003). Common types of infections that are diagnosed and treated by physicians include wound infections, urinary tract infections, upper respiratory tract infections, pneumonia, and intestinal tract infections. All of these can range in severity due to the specific organism, the patient's status (co-existing conditions, age, etc.), and whether or not treatment is received in a timely fashion.

SIRS, Sepsis, Severe Sepsis, and Septic Shock

The 1992 American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) Consensus Conference convened to "to provide a conceptual and practical framework to define the systemic inflammatory response to infection, which is a progressive injurious process that falls under the generalized term 'sepsis' and includes sepsis-associated organ dysfunction as well" (Levy et al., 2003). Prior to this point, the terms sepsis, sepsis syndrome, and septic shock were used interchangeably when referring to various points on the continuum of the infection process (from signs and symptoms of infection to septic shock and organ dysfunction). The definitions for systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock were developed and later reviewed and revised by the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference sponsored by the SCCM, ACCP, the European Society of Intensive Care Medicine (ESICM), the American Thoracic Society, and the Surgical Infection Society. The revised terms are widely used today by physicians to make clinical decisions but are not diagnostic in nature. The terms only provide a means for identifying which specific criteria that

المنارة للاستشارات
www.manaraa.com

patients meet when they are suspected of having a systemic response to infection (Levy et al., 2003; Nystrom, 1998; Rangel-Frausto, 2005).

The term systemic inflammatory response syndrome (SIRS) was introduced to describe the activation of the immune response system by localized or generalized infection, trauma, thermal injury, or sterile inflammatory processes. SIRS can be initiated by both infectious and non-infectious conditions. Patients with SIRS are defined as having two or more of the following clinical findings:

- Body temperature of $>38^0$C or $<36^0$C
- Heart rate >90 beats per minute
- Respiration rate >20 breaths per minute
- PCO_2 <32 mm Hg (normal approximately 35 – 45 mmHg)
- White blood cell (WBC) count $>12.0 \times 10^3$ cells/mm^3 of blood , $<4.0 \times 10^3$ cells/mm^3 of blood, or >10% bands (immature cells)

However, these criteria are too non-specific to be useful in identifying a specific cause (infectious vs. non-infectious) for the activation of the immune response (Levy et al., 2003).

Sepsis was defined by the 2001 conference as SIRS in the presence of a confirmed infection. Infection resulting in sepsis sets off a cascade of inflammatory processes that lead to activation of the coagulation system. This eventually leads to alterations in microvascular circulation that result in organ dysfunction. Positive identification of an infection can be a difficult and lengthy process. Therefore, if one or

المنارة للاستشارات
www.manaraa.com

more of the following conditions and SIRS are present, a presumptive diagnosis of sepsis can be made:

- Significant edema or positive fluid balance (fluid intake greater than fluid output of 20 mL/kg over 24 hrs or more)
- Hyperglycemia (plasma glucose >120 mg/dL) in the absence of diabetes
- Biomarkers of acute inflammation: plasma C-reactive protein >2 SD above the normal value or plasma procalcitonin >2 SD above the normal value
- Mixed venous oxygen saturation (SVO2) >70% (measured from a pulmonary artery, with a high value indicative of low oxygen demand resulting from such causes as hypothermia, anesthesia, pharmacologic paralysis, and sepsis
- Cardiac index >3.5 L/min/M^2 (normal amount of blood pumped while the body is "at-rest" by the ventricles per minute relative to body size is 3 L/min/M^2)

These criteria are signs of potential early organ failure that may trigger a physician to think that a patient "looks" septic (Edwards, 1991; Khalili et al., 2010; Levy et al., 2003; Mohrman & Heller, 2006).

Severe sepsis is defined as sepsis with organ dysfunction. Organ dysfunction, hypoperfusion, and hypotension variables denoting septic shock include (but are not limited to):

- Arterial hypoxemia (PaO2/fraction of inspired oxygen [FiO2] ratio of <300 torr – normal values are >400; <300 is indicative of acute lung injury)
- Acute oliguria (urine output <0.5 mL/kg/h for at least 2 h)
- Creatinine >0.5mg/dL increase above baseline

المنارة للاستشارات
www.manaraa.com

- Coagulation abnormalities (international normalized ratio >1.5 or activated partial thromboplastin time >60 sec)
- Thrombocytopenia (platelet count <100,000/mm^3)
- Hyperbilirubinemia (plasma total bilirubin >4 mg/dL or 70 mmol/L)
- Hyperlactatemia (>1 mmol/L)
- Arterial hypotension (systolic blood pressure <90 mmHg, mean arterial pressure (MAP) <70 mmHg (calculation based upon systolic and diastolic values; the average arterial blood pressure during a single cardiac cycle and normally 70 – 110 mmHg), or a systolic blood pressure decrease >40 mmHg from baseline)
- Altered mental status

These parameters represent one level of organ dysfunction. The degree of organ dysfunction may be defined by using the Sequential Organ Failure Assessment (SOFA). SOFA is a six-organ dysfunction/failure score that measures the function of the lungs, coagulation, liver, heart, central nervous system, and kidneys. When using the SOFA system, each organ is graded on a scale of 0 – 4 depending upon the level of the parameter being assessed. Patients with severe sepsis exhibit a level of organ dysfunction that corresponds roughly with SOFA level of two for each organ system (Levy et al., 2003; Mohrman & Heller, 2006; Nystrom, 1998).

Septic shock is a state of acute circulatory failure that is characterized by hypotension even with adequate resuscitation with fluids and is unexplained by any other cause. The 2001 Consensus Conference (Levy et al., 2003) defined the level of hypotension in septic shock using the following levels:

المنارة للاستشارات
www.manaraa.com

- Systolic arterial pressure < 90 mm Hg
- Mean arterial pressure (MAP) <60 mm Hg
- A reduction in systolic blood pressure >40 mm Hg from baseline

For physicians who have to identify patients with SIRS, sepsis, severe sepsis, and septic shock, these definitions still present difficulties since none of the criteria in the current definitions are specific for infection or sepsis. Physicians still resort to the clinical impression that "he/she looks septic" then looks for a source of infection by ordering various cultures and tests to identify a causative agent (Levy et al., 2003).

ALF has many clinical similarities to septic shock leading to the suspicion that they share pathogenic mechanisms that lead to the progression from SIRS to multiple organ dysfunction. ALF patients are prone to infection due to loss of the protective effects of the liver further confusing the picture: "What is ALF without sepsis?" and "What is sepsis in ALF?". Studies have shown that the presence of SIRS in ALF is associated with progression to hepatic encephalopathy and that the hemodynamic profile in ALF patients is similar to that seen in septic shock. While the systemic inflammatory profile is well understood in sepsis and septic shock, this is not the case in ALF (Antoniades et al., 2008; Rolando et al., 2000; Vaquero et al., 2003).

While SIRS and sepsis in ALF have not been well studied to date, ALF patients do have an increased risk of infection and subsequently developing sepsis and sepsis-induced organ failure (Rolando et al., 2000; Wade et al., 2003; Stravitz, 2008). There is much confusion between signs and symptoms of ALF and signs and symptoms of sepsis. The overlap pertains to the fact that the diagnosis of sepsis requires the finding of a

المنارة للاستشارات
www.manaraa.com

positive blood culture, which is often delayed or not detected, even in cases where presumptive evidence of bacterial infection is very strong. It has been estimated that as many as 80% of ALF patients develop bacterial infections. The most common types of infections include pneumonia (50%), urosepsis (22%), IV catheter-induced bacteremia (12%), and spontaneous bacteremia (16%). Infection is associated with progressive worsening of HE, and is one of the most common causes of multi-organ dysfunction and death in ALF (Stravitz, 2008; Vaquero et al., 2003).

There are multiple reasons for this susceptibility to infection. Patients with acute liver failure have immunological defects that include impaired function of the white blood cells and decreased activity of plasma proteins, including components of the complement cascade. ALF patients are also subject to an increased use of invasive procedures that are associated with increased risk of infection. Such procedures include insertion of intravenous lines, monitors for intracranial pressure and arterial pressure, urine catheters, and mechanical ventilators. Cerebral edema increases the chance of lung infection because chest physiotherapy and bronchial suction are contraindicated.

Methods to Identify Bacterial Organisms

Diagnosis of bacterial infection by the microbiology laboratory is a time consuming multi-step process. The process of identifying an infection begins by choosing an appropriate specimen, proper collection of the specimen to avoid contamination, prompt transportation to the laboratory, and providing the laboratory with appropriate information concerning the specimen and the patient. Once the specimen is in the laboratory, the historical approach to identifying bacterial pathogens involved inoculating

المنارة للاستشارات
www.manaraa.com

the specimen onto appropriate culture medium and incubating the culture specimen for 24 to 48 hours. If bacteria grow, isolated colonies are chosen to subculture for identification and antibiotic susceptibility testing, requiring an additional 24 hours. The total time required to identify the offending organism and determine its antimicrobial susceptibility profile is typically 48 to 72 hours (Levinson, 2010).

In some cases, additional time is required because patients have previously received antibiotics or the infectious organisms are slow growing. Some organisms will not grow using standard culture techniques, require special media or other growth conditions, and some bacteria do not survive the collection and transport process (Fenollar & Raoult, 2007; Peters, van Agtmael, Danner, Savelkoul, & Vandenbroucke-Grauls, 2004; Schrenzel, 2007; Sixou, 2003; Stefani, 2009). Despite these drawbacks, culture remains the gold standard for the detection of bacteria in patients with infections. Cultures have the advantage because they can identify both organisms that are suspected and those that are unsuspected as being the cause for infections (Sixou, 2003).

In the past 10 to 20 years, new methods have been developed to identify bacteria causing infections. These methods include polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), DNA-based microarray, mass spectroscopy, and immunoassay. FISH and PCR techniques have been shown to decrease the time required to identify bacteria from blood culture samples (Peters et al., 2004; Stefani, 2009). PCR has been shown in multiple studies to be able to identify bacteria directly from blood culture bottles (Gebert, Siegel, & Wellinghauser, 2008; Jaffe, Lane, Albury, & Niemeyer, 2000; Kilic, Muldrew, Tang, & Basustaoglu, 2010; Ruimy et al., 2008;

المنارة للاستشارات
www.manaraa.com

Stefani, 2009), from culture swabs (Francois et al., 2003), from cerebral spinal fluid (du Plessis, Smith, & Klugman, 1998), and from colonies already isolated in culture (du Plessis et al., 1998; Geha, Uhl, Gustaferro, & Persing, 1994; Georghiou et al., 1995; Hsueh et al., 1998; Sabet et al., 2006; Sarwari et al., 2004). Mass spectroscopy, while not commonly used, has been shown to identify bacteria accurately from isolates and clinical samples (Ho & .Reddy, 2010). Microarray techniques have been used to identify bacteria both from isolates (Strommenger et al., 2007) and from blood culture bottles (Tissari et al., 2010). Many of these technologies can identify specific bacterial species and strains that have known susceptibility patterns. While this does not eliminate the need for antimicrobial sensitivity studies, it may improve the process of identifying appropriate antibiotic therapy in some cases (Francois et al., 2003; Ruimy et al., 2008; Stefani, 2009; Strommenger et al., 2007; Tissari et al., 2010).

All of these methods show promise by decreasing the time required to identify bacteria from clinical specimens, but major drawbacks. In all cases, you can only “find what you are looking for” (Sixou, 2003). The probes required for the FISH, PCR, and microarray techniques are pre-determined. The selected probes will test for multiple bacterial species and strains but multiple arrays must be used to expand the range of organisms that can be detected. Mass spectroscopy requires either pure culture isolates or significant pre-treatment of samples to identify organisms in complex specimens (Ho & Reddy, 2010). PCR techniques are susceptible to laboratory contamination and background bacterial DNA from either dead organisms or from organisms that are present but not responsible for the infection (Stefani, 2009).

المنارة للاستشارات
www.manaraa.com

Procalcitonin

Procalcitonin is the precursor form of calcitonin, a hormone whose primary source is the parafollicular C cells of the thyroid gland. Calcitonin has a hypocalcemic and hypophosphatemic effect, inhibiting bone resorption, and stimulating the kidney to excrete phosphorus, calcium, and sodium. Calcitonin can be used as a biomarker of medullary thyroid cancer (MTC). However, the mechanisms by which calcitonin produces the above effects in the human body are not known. The removal of the thyroid, gland such as for treatment of MTC, does not cause a disruption in calcium hemostasis (Becker, Nylen, White, Muller, & Snider, 2004; Bracq et al., 1993; Carrol, Thomson, & Hart, 2002; Muller & Becker, 2001).

PCT is one of a group of peptides in the calcitonin super-family of peptides. Other peptides in the group include calcitonin gene-related peptide I, II (CGRP-I, CGRP-II), amylin, and andrenomedullin. The PCT peptide has an approximate molecular weight of 14.5 kDa and consists of a sequence of 116 amino acids. Both PCT and CGRP-I are encoded by the Calc-1 gene located on chromosome 11p15.4 (Christ-Crain & Muller, 2008; Jin & Khan, 2010; Russwurm, Oberhoffer, Zipfelk, & Reinhart, 1999). The peptide has three regions (Figure 1): the PCT amino terminus, the mature calcitonin segment, and the carboxyl-terminus called katacalcin (Carrol et al., 2002).

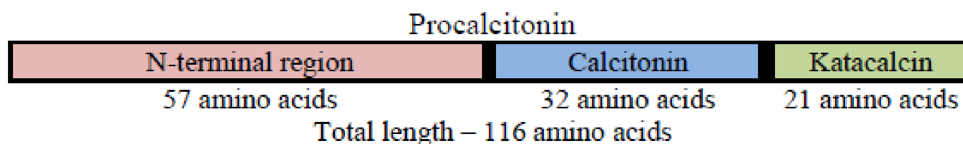

Figure 1: Schematic of Procalcitonin Molecule

Figure was derived from E.D. Carrol et al., (2002) International Journal of Antimicrobial Agents, 20: 1-9.

www.manaraa.com

In the absence of infection, the production of PCT outside of the neuroendocrine cells of the thyroid and the lung is suppressed. In the presence of sepsis, all tissues produce PCT. Because of this dual role, PCT is considered a "homokine". Homokines can either act as a hormone as in the normal physiologic state or as a cytokine during inflammatory processes (Muller & Becker, 2001; "PCT in patients with sepsis", n.d.). To date no other causes of PCT increase has been determined.

The factors mediating the production of homokines are as yet unknown. It may be induced either by toxins produced by bacteria or by humoral- or cell-mediated host response. As with other cytokines, there is little intracellular storage of PCT during sepsis. While synthesis of PCT is necessary for the production of calcitonin, animal studies have shown that increased concentrations of PCT may have lethal effects during sepsis. Administration of PCT to septic hamsters with peritonitis doubled the death rate to over 90%. Immunoneutralization of PCT by the administration of antiserum in septic hamster and pig studies led to increased survival of these animals (Muller & Becker, 2001; Nylen et al., 1998; Nystrom, 1998; Whang et al., 1999).

Procalcitonin Testing

Most PCT assays have been developed in cooperation with BRAHMS Diagnostic GmbH. BRAHMS placed the first PCT assay into use in 1996, with a rapid test following in 1999. The FDA approved the use of the manual BRAHMS PCT LIA (formerly LUMItest PCT) in the US in 2005. The BRAHMS PCT LIA, ("Immunoluminometric assay (ILMA) for the determination of PCT", 2005) is a dual monoclonal antibody luminescence immunoassay. The assay uses a coated tube technique in which the anti-

المنارة للاستشارات
www.manaraa.com

calcitonin capture antibody is immobilized on the inner surface of the tube and a second anti-calcitonin antibody is labeled with a luminescent acridine derivative. During the incubation phases of the procedure, PCT is “sandwiched” between the two calcitonin antibodies. The amount of PCT in the sample is determined by measuring the luminescence signal using a luminometer when Basiskit LIA reagents are added to the tube. The intensity of the luminescence signal (RLUs, relative light units) is directly proportional to the PCT concentration. The assay takes two to three hours to complete, requires standards and controls to be analyzed with each run, and is usually performed in batch mode ("Immunoluminometric assay (ILMA) for the determination of PCT (coated tube system*)*", 2005; McGee & Baumann, 2009; Steinbach et al., 2004).

The first automated PCT assay was the BRAHMS KRYPTOR assay. The KRYPTOR assay received FDA clearance for use in the US in March 2008. This assay uses Time Resolved Amplified Cryptate Emission (TRACE) technology, with a non-radiative transfer of energy. The PCT molecule is sandwiched between a PCT antibody tagged with europium cryptate (donor) and a PCT antibody tagged with XL665 (acceptor) forming an immune complex. When a pulsed nitrogen laser at 337 nm excites the sample, the donor emits a fluorescent signal at 620 nm with an excited state $t_{1/2}$ in the milli-second range. The acceptor emits a fluorescent signal at 665 nm with an excited state half-life ($t_{1/2}$) in the nano-second range. If the donor and acceptor are bound in an immune complex, the fluorescent signal of the acceptor is amplified, and its $t_{1/2}$ is prolonged to the microsecond range by resonance energy transfer from the donor. The signal intensity at 665 nm is proportional to the amount of PCT in the sample. The assay

www.manaraa.com

calibration is good for seven days and results are available in 25 – 40 minutes ("BRAHMS PCT sensitive KRYPTOR", 2008; McGee & Baumann, 2009; Steinbach et al., 2004).

The LIA (LUMItest) and the Kryptor assays have been utilized for most of the clinical studies examining the clinical utility of measuring PCT to date. A multi-center study compared the two assays for assay characteristics including within- and between-run estimates of precision, linearity, functional assay sensitivity, and a comparison of PCT values on patients' samples. The study found that the main differences between the assays were that the LUMItest, being a manual assay, required more time to obtain results. Furthermore, the functional sensitivity of the LUMItest was higher (0.30 ng/mL, although some studies have shown it to be as low as 0.1 ng/mL) than that of the KRYPTOR assay (0.04 ng/mL). The functional assay sensitivity is the lowest concentration of PCT that can be analyzed with a coefficient of variation (CV) less than or equal to 20%. The reference range for the two assays differs due to the functional sensitivities. The reference range for normal (non-infected) subjects is <0.3 ng/mL for the LUMItest assay and <0.05 ng/mL for the KRYPTOR assay. The analytical measurement range for the KRYPTOR assay extends only to 50 ng/mL, while the LUMItest assay can measure PCT concentrations up to 500 ng/mL. However, the instrumentation for the KRYPTOR assay has automatic dilution protocols that increase the measurement range up to 5000 ng/mL. The within-run and between-run precision of the KRYPTOR assay was superior to the LUMItest assay. Within-run precision CVs were 3.14% - 4.56% (at concentrations of 18.3 ng/mL and 0.26 ng/mL, respectively) for KRYPTOR vs. 3.14% –

المنارة للاستشارات
www.manaraa.com

6.83% (at concentrations of 1.69 ng/mL and 15.70 ng/mL, respectively) for LUMItest and between-run was 5.09% - 8.34% (at concentrations of 3.40 ng/mL and 9.18 ng/mL, respectively) for the KRYPTOR and 6.28% - 16.44%, (at concentrations of 15.77 ng/mL and 0.32 ng/mL, respectively) for the LUMItest. The highest correlation between the LUMItest and the KRYPTOR assays was in the 0 – 50 ng/mL range, which is the common measurement range for both assays with undiluted specimens. Linear regression analysis on PCT results obtained on patients' samples demonstrated r = 0.92 in the 0.3 – 50 ng/mL range (n = 534) and r = 0.91 for all data (n = 696). The conclusion was that there should be no clinically relevant difference between PCT values obtained by the two assays (Steinbach et al., 2004).

Siemens Healthcare Diagnostics has developed the BRAHMS PCT assay for their ADVIA Centaur Immunoassay Analyzer. The assay has not yet been approved for use in the US by the FDA but has been approved by the European Union (EU) regulatory agency for use in Europe, Australia, and Canada. The assay is a one-pass antibody sandwich immunoassay that uses an anti-fluorescein isothiocyanate-labeled ((FITC) monoclonal antibody covalently bound to paramagnetic particles (PMP), two fluorescent capture monoclonal antibodies, and an acridinium ester (AE)-labeled monoclonal antibody. The PCT molecule is sandwiched between fluorscein labeled antibodies and acridinium ester labeled antibodies with the whole complex held to an anti-fluorscein labeled paramagnetic particle (solid phase structure). When the acridinium labeled complex is exposed to acid and then base reagents a chemiluminescent reaction occurs which produces a flash of light that is measured in RLUs. The RLUs are directly

www.manaraa.com

proportional to the amount of PCT in the serum ("Advia centaur immunoassay system reference manual", 2003; "Advia centaur and advia centaur XP systems - procalcitonin (PCT)", 2010).

The ADVIA Centaur BRAHMS PCT (CenPCT) has been compared to the BRAHMS KRYPTOR assay in a 600 patient study in which single replicate serum samples were assayed using each method. The specimen concentration ranged from 0.01 ng/mL to 573 ng/mL. Regression analysis of the results produces a correlation coefficient (r) of 0.993 and a slope of 1.00 and an intercept of -0.04. Further studies produced a functional assay sensitivity of <0.05 ng/mL. A normal population study resulted in an average PCT value of 0.02 ng/mL (Per package insert the normal range is reported as <0.1 ng/mL.). The analytical range of the CenPCT is 0.02 – 75.0 ng/mL. The ADVIA Centaur has an automatic dilution protocol that allows for an increase in the measurement range of up to 1440 ng/mL. The within-run precision CVs for the CenPCT were 1.1% - 6.1% (at concentrations of 33.77 ng/mL and 0.06 ng/mL, respectively). The between-run precision CVs for the CenPCT were 3.3% - 8.6% (at concentrations of 33.77 ng/mL and 0.06 ng/mL, respectively) (Aso, Baker, Freeman, & Navarro, 2009). Table 1 shows a summary of the BRAHMS KRYPTOR and ADVIA BRAHMS PCT assays.

PCT as a Biomarker of Bacterial Infection or Sepsis

Assciot. Gendrel, Carson, Raymond, Guilbaud, and Bohuon (1993) first suggested procalcitonin could be a marker of bacterial infection in 1993 when they found increased concentrations of PCT in pediatric patients with bacterial infection (Bohuon, 2000; Carrol et al., 2002) . In a subsequent study by Dandonna in 1994, healthy volunteers received a

المنارة للاستشارات
www.manaraa.com

Table 1: KRYPTOR vs. ADVIA Centaur Assays for SPCTC

	BRAHMS KRYPTOR PCT	ADVIA Centaur BRAHMS PCT
Functional sensitivity	0.04 ng/mL	<0.05 ng/mL
Normal population	<0.05 ng/mL	0.02 ng/mL
Reference range	<0.05 ng/mL	<0.1 ng/mL
Analytical range	0.05 – 50 ng/mL	0.02 – 75 ng/mL
Range with auto-dilution	5000 ng/mL	1440 ng/mL
Within run CVs	3.14% - 4.56%	1.1% - 6.1%
Between run CVs	5.09% - 8.34%	3.6% - 8.6%

bolus of endotoxins after which SPCTCs were measured at defined intervals. PCT concentrations had started to rise by three hours, reached a plateau by six hours, and remained at these levels for up to 24 hours (Bohuon, 2000; Carrol et al., 2002).

In a case report of a 76 year old female who received an intravenous (IV) solution contaminated (probably resulting from improper storage of the IV solution between dose administrations) with *Acinetobacter baumanii*, PCT rapidly increased in the blood following development of systemic infection (Brunkhorst, 1998). The PCT was undetectable at 1.5 hours after the injection, became detectable at 2.5 hours, and peaked at 13.5 hours. Serial measurements of PCT during follow-up days determined that the PCT half-life is 22.5 hours (Brunkhorst, Heinz, & Forycki, 1998).

In a study in which seven healthy volunteers were administered intravenous solutions containing endotoxins from *Escherichia coli* 0113:H10:k, PCT was not detectable at two hours post injection but was detectable in all samples at 4 hours. Values peaked between 8 - 12 hours post injection and remained high at 24 hours (Dandona et al., 1998).

المنارة للاستشارات
www.manaraa.com

Studies examining the elimination rate of PCT indicate that the approximate half-life of PCT is 30 hours for patients with normal renal function. The median PCT half-life in patients with renal dysfunction was 33.1 – 44.7 hours. While patients with renal dysfunction may have a longer PCT half-life, the kidneys do not appear to be the major pathway of PCT elimination from the blood (Meisner, Schmidt, Huttner, & Tschaikowsky, 2000; Meisner, Huettemann, Schmidt, Hueller, & Reinhart, 2001). These results would suggest that PCT can be used to identify bacterial-induced systemic inflammation.

Studies to demonstrate the clinical utility of PCT as a biomarker of bacterial infection have taken two approaches: 1) use of PCT as a diagnostic biomarker of infection and 2) use of PCT to confirm the appropriateness of specific antibiotic therapy and patient outcomes. Studies of PCT as a diagnostic biomarker of infection have looked at patients with respiratory tract infections, peritonitis, meningitis, and post-surgical infections in the setting of the emergency department (ED), intensive care unit (ICU), and general hospital wards. There have been at least nine randomized trials (seven single-center and two multi-center) looking specifically at the use of PCT to guide antibiotic therapy.

Several investigators have examined the utility of measuring PCT in patients that present to the ED, ICU, and general hospital wards with signs of infection or sepsis. In two studies, subjects were admitted to the ICUs for their respective hospitals with a suspected diagnosis of infection. The studies classified the subjects based upon their SIRS status into one of four groups: SIRS, sepsis, severe sepsis, or septic shock. In a

www.manaraa.com

study by Brunkhorst, Wegscheider, Forycki, & Brunkhorst (2000), a PCT cutoff of 2.0 ng/mL predicted severe sepsis with an estimated 96% sensitivity and 86% specificity using receiver operator curve (ROC) analysis. A cutoff of 11.6 ng/mL predicted septic shock with an estimated 53% sensitivity and 72% specificity. Mean PCT values for the groups were 0.41 ng/mL (SIRS), 0.53 ng/mL (sepsis), 6.91 ng/mL (severe sepsis), and 12.89 ng/mL (septic shock). A study by Harbarth, et al. (2001) had similar results. Investigators determined that a PCT cutoff of 1.1 ng/mL predicted sepsis with 97% sensitivity and 78% specificity using ROC analysis. The mean PCT values for the groups were 0.6 ng/mL (SIRS), 3.5 ng/mL (sepsis), 6.2 ng/mL (severe sepsis), and 21.3 ng/mL (septic shock).

Cheval, C., Timsit, J. F., Garrouste-Orgeas, M., Assicot, M., De Jonghe, B., Misset, B., et al. (2000) evaluated the use of PCT to assess infection in the medical/surgical ICU setting. The study included four groups of acutely ill patients admitted to the ICU. Sixty patients were assigned to one of four groups: 1) shock (with bacterial infection), 2) non-septic shock (with no bacterial infection), 3) infected, and 4) control (no evidence of infection). Patients with proven bacterial infection (groups 1 – 3) had mean PCT concentrations of 72 ng/mL which was significantly higher than the PCT results for patients in the control group (Group 4) at 2.9 ng/mL (p-value = 0.0003). Patients with shock and a confirmed bacterial infection (Group 1) had higher mean PCT values than those with shock and no confirmed infection (Group 2), 89 ng/mL and 2.9 ng/mL, respectively (p-value = 0.0004). A SPCTC of at least 20 ng/mL predicted positive

المنارة للاستشارات
www.manaraa.com

blood cultures in infected patients with sensitivity of 89 ± 21% and specificity of 83 ± 15%.

A study by Guven, H., Altintiop, L., Baydin, A., Esen, S., Aygun, D., Hokelek, M., et al. (2002) enrolled 34 patients that were admitted to the ED between January 1999 and September 2000, who were at risk for sepsis due to hospitalization or insertion of intravascular and/or urinary catheters. All patients met at least two criteria for SIRS. The patients were divided into two groups: sepsis not suspected and sepsis suspected. The sepsis not suspected group included 15 patients who had minor infections (upper respiratory tract, otitis media, or gastroenteritis) who were observed in the ED for 24 hours and then discharged. The sepsis suspected group included 19 patients with a history of previous cerebrovascular disease, chronic obstructive pulmonary disease, diabetes mellitus, or previous hospitalizations but had no documented bacterial infection. The patients in the sepsis not suspected group all had negative blood cultures and an average SPCTC of 0.23 ng/mL. In the sepsis suspected group, 13 of 19 patients had positive blood cultures and an average PCT value of 67.89 ng/mL. Two patients with negative blood cultures had near normal PCT values (normal 0 – 5 ng/mL) while two others had extremely high values (approximately 500 ng/mL) and were diagnosed with acute pancreatitis (Guven et al., 2002).

In a study by Brunkhorst, Eberhard, & Brukhorst (1999), 27 patients with adult respiratory distress syndrome (ARDS) were prospectively studied to determine the ability of PCT to discriminate between septic and non-septic causes of ARDS: 10 patients developed ARDS due to injuries to the lung and 17 developed ARDS due to bacterial

المنارة للاستشارات
www.manaraa.com

infection. Serum PCT concentrations were measured every four to six hours for 72 hours immediately following a diagnosis of ARDS. PCT values in patients with non-septic ARDS averaged approximately 1.0 ng/mL (with no values higher than 2.9 ng/mL), while the PCT values in septic ARDS patients were all greater than 5.0 ng/mL (Brunkhorst et al., 1999).

In a study by Luyt, C., Guerin, V., Combes, A., Trouillet, J., Ayed, S. B., Bernard, M., et al. (2005), 63 prospectively enrolled patients with ventilator-associated pneumonia (VAP) were studied to determine if PCT kinetics could serve as a prognostic marker of infection in these patients. In the study, 38 patients had unfavorable outcomes (death, VAP recurrence, or extrapulmonary infection requiring antibiotics before Day 28) and 25 patients had favorable outcomes (not meeting one of the unfavorable outcome requirements). Median PCT values were significantly higher for unfavorable outcome group than for the favorable outcome group on days 1, 3, and 7. On Day 1, a PCT value >1.0 ng/mL had an odds ratio of 12.3 of being associated with an unfavorable outcome. Later time points demonstrated increasing odds ratios with an odds ratio of 24.6 on day 3 for a PCT > 1.5 ng/mL and 64.2 on day 7 for a PCT > 0.5 ng/mL (Luyt et al., 2005).

Reith, Mittelkotter, Wagner, & Thiede (2000) assessed serum PCT as a measure of the severity in patients with septic abdominal illnesses. In this prospective study, serial measurements of PCT were carried out in 246 patients with infective or septic episodes and in 66 patients with elective operations who served as controls. The causes of infective peritonitis in this study included pancreatitis, colon perforation, abscess, trauma, and mesenteric infarction. In the peritonitis group, 59 of the 246 patients died. The mean

المنارة للاستشارات
www.manaraa.com

PCT value in patients who died rose from 4.2 ng/mL initially, to 13.2 ng/mL at time of death. The remaining peritonitis subjects had a mean initial PCT value of 2.1 ng/mL that rose to 4.8 ng/mL on day 4 then decreased to 0.4 ng/mL at end point (day 10 post-operatively, 3 months post-operatively for patients with metastasis, or death). PCT values for the control group who had no signs of post-operative infection or sepsis were within the reference range (0.1 – 0.8 ng/mL) for 98% of the samples tested (424 of 431 samples). The remaining seven samples had values between 0.8 and 1.2 ng/mL. The results of this study indicated that PCT was elevated in septic patients with peritonitis and that a reduction of PCT during their clinical course was predictive of survival (Reith et al., 2000).

PCT has been shown to be a strong predictor for distinguishing bacterial and non-bacterial meningitis (such as viral, fungal, drug related, and tick-borne disease related). In a study by Dubos, et al. (2008), data from 198 children admitted to six pediatric ED or ICU units in five European countries were retrospectively examined to determine the usefulness of PCT as a marker of bacterial meningitis. At a 0.5 ng/mL threshold, PCT had 99% sensitivity and 83% specificity for distinguishing between bacterial and aseptic meningitis. Ninety-six of the 198 patients studied had bacterial meningitis confirmed by a positive culture but only 75% had a positive gram stain report. The bacterial organisms identified in these patients included *Neisseria meningitides, Streptococcus pneumoniae, H influenzae,* and *Streptococcus pneumoniae*. The median PCT value in patients with bacterial meningitis was 21.5 ng/mL while the median PCT value in patients with non-bacterial meningitis was 0.3 ng/mL (p-value $< 1 \times 10^{-6}$) (Dubos et al., 2008).

المنارة للاستشارات
www.manaraa.com

In two studies of post-operative patients, PCT was examined as a biomarker of infection post surgery. Falcoz, P.-E., Laluc, F., Toubin, M.-M., Puyraveau, M., Clement, F., Mercier, M., et al. (2005) studied patients after thoracic surgery and found a mean PCT value in 132 non-infected patients of 0.63 ng/mL versus 3.6 ng/mL for 25 patients who developed a post-operative infection (p-value = 0.0001). Meisner, Tschaikowsky, Hutzler, Schick, & Schuttler (1998) studied 130 patients with different types of surgeries, measuring PCT pre-operatively and for five days post-operatively. Patients were assigned to one of five groups based upon the type of surgery performed: 1) minor surgery - hip replacement, peripheral vascular surgery, and general surgery, 2) minor abdominal surgery - cholecystectomy, 3) abdominal surgery of the intestine - resection of the colon, sigma, and rectum, and gastrectomy, 4) major abdominal or thoracic surgery - esophagectomy, Whipple procedure, and major vascular surgery, and 5) cardiac and thoracic surgery - coronary artery bypass, resection of the lung, and diagnostic thoracotomy. For patients with a normal post-operative course without infection or sepsis (n = 117), the median PCT values for all groups were < 1.0 ng/mL except the surgery of the intestine (group three) that had a median value of 1.5 ng/mL for patients with a normal post-operative course without infection or sepsis. Thirteen patients (a heterogeneous mix of the abdominal surgery of the intestine, major abdominal or thoracic, and cardiac and thoracic surgery groups) had abnormal post-operative courses. An abnormal post-operative course was defined as fulfilling SIRS or sepsis criteria, any clinical sign of infection, increased body temperature above 38^0C after post-op day 2, extubation after post-op day 1, requiring catecholamine therapy, cardiac insufficiency,

www.manaraa.com

pulmonary infiltrates, local wound infection, or reoperation. This group had a median PCT value of 6.48 ng/mL while only one patient had an identified bacterial infection (Meisner et al., 1998). Both studies showed a general increase in PCT immediately following surgery. The amount of increase observed was dependent upon the type of surgery performed. However, in both studies, the PCT level was lower in patients who did not develop an infection or sepsis post-operatively. The cause of the higher PCT values in the non-septic patients (from both studies) is not entirely understood. Both authors noted these increases and speculated that they may be a result of the type of surgery (the majority of the surgeries in these patients were cardiac and thoracic in nature) and due to lung involvement. An increase in PCT after lung injury in the absence of infection has been noted (Falcoz et al., 2005; Meisner et al., 1998).

The above studies indicated that PCT is a good predictor of bacterial infection or sepsis in most cases. The studies also showed that PCT was not an absolute indicator of infection and should be used in conjunction with other infection indicators.

PCT Use to Guide Antibiotic Therapy

Not only is serum PCT concentration measurement being used as an indicator of infection, but it is also being used to guide antibiotic treatment. Studies have shown that PCT-guided therapy has substantially reduced the amount of antibiotic used by decreasing the prophylactic use of antibiotics when a bacterial infection is suspected but not actually present and shortening the length of antibiotic treatment in bacterial infections that respond to therapy. The reduced number of antibiotic treatment days lead to fewer antibiotic adverse effects without increasing the number of adverse events

المنارة للاستشارات
www.manaraa.com

(Bouadma et al., 2010; P. E. Charles et al., 2009; Christ-Crain et al., 2004; Schuetz, Christ-Crain, & Muller, 2009; Tang, Huang, Jing, Shen, & Cui, 2009).

Studies have compared standard of care (SOC) antibiotic therapy to PCT guided antibiotic therapy in patients with lower respiratory tract infections (LRTI). Diagnoses that are related to LRTI include acute bronchitis, acute exacerbations of chronic pulmonary disease, asthma, and pneumonia. Patients presenting with signs and symptoms of LRTI are often treated with antibiotics, without clear evidence of a bacterial infection. LRTIs are mainly viral in nature. LRTI is the leading cause for antibiotic prescriptions in the Western hemisphere and contributes to increasing bacterial resistance to antibiotics (Christ-Crain et al., 2004; Schuetz et al., 2009; Schuetz et al., 2009; Schuetz, Christ-Crain, & Muller, 2009).

Christ-Crain, et al. (2004) conducted a prospective randomized controlled single blinded intervention trial with 243 subjects enrolled in the study. The subjects were randomly assigned to either the SOC (standard antimicrobial therapy) or PCT guided groups. In the PCT group, the physician first had to declare his/her intention to prescribe antibiotics after which they were advised to follow the PCT algorithm for prescribing antibiotics. The algorithm using the serum PCT concentration was:

- 0.1 ng/mL or less indicates absence of bacterial infection and antibiotics are strongly discouraged
- 0.1 – 0.25 ng/mL indicates bacterial infection is unlikely and antibiotics are discouraged

المنارة للاستشارات
www.manaraa.com

- 0.25 – 0.5 ng/mL indicate possible bacterial infection and the doctor is advised to initiate antibiotic therapy
- 0.5 ng/mL or greater is suggestive of a bacterial infection and antibiotic treatment is strongly suggested.

For patients on antibiotics at the time of hospital admission, discontinuation of antibiotic therapy was recommended if the PCT value was less than 0.25 ng/mL (Christ-Crain et al., 2004).

The second study is the Procalcitonin Guided Antibiotic Therapy and Hospitalization in Patients with Lower Respiratory Tract Infections: The ProHOSP Study. This was an investigator initiated multi-center randomized controlled trial with six participating tertiary care hospitals in Switzerland. Patients were randomized into PCT-guided treatment group using the PCT cut-off ranges described above or SOC treatment to initiate or stop antibiotic treatment (Schuetz et al., 2009).

Both studies found that the duration of antibiotic therapy was significantly less in the PCT-guided antibiotic therapy groups compared to the SOC groups (Table 2) with no difference in the rate of adverse outcomes (death). The ProHOSP study also determined that the PCT-guided treatment group had fewer adverse effects (including nausea, diarrhea, and rash) from antibiotics than SOC (133 vs. 193 respectively), a decrease of 28.2% (Christ-Crain et al., 2004; Schuetz et al., 2009).

The Procalcitonin to Reduce Antibiotic Treatments in Acutely ill patients (PRORATA) trial was a prospective parallel group study undertaken in France to assess critically ill patients with suspected bacterial infections. The study was a multi-center

المنارة للاستشارات
www.manaraa.com

Table 2: Published Studies of PCT Guided Antibiotic Therapy vs. SOC

Antibiotic Treatment Group			
	SOC	PCT guided	Percent difference
Christ-Crain			
# patients	119	124	
Mean days antibiotic treatment	12.8	10.9	-14.8%
Died	4	4	0%
ProHOSP			
# patients	688	671	
Mean days antibiotic treatment	8.7	5.7	-34.8%
Died	33	34	3.0%
PRORATA			
# patients	314	307	
Mean days antibiotic treatment	9.9	6.1	-38.4%
Died	64	65	1.6%

study of patients in the medical and surgical ICUs of five university-affiliated hospitals and one general hospital. All adults with suspected bacterial infections in the ICUs were eligible for the study if they had not been receiving antibiotics before the study. Patients were randomly assigned to either a PCT-guided antibiotic therapy group or a SOC group. The results of this study (see Table 1) were similar to the previously described studies in that the PCT-guided therapy group - shorter average number of days of antibiotic exposure without affecting the rate of adverse outcomes (Bouadma et al., 2010). Table 1 summarizes the results from the three aforementioned studies.

In an observational study by Charles et al., (2009) of 180 patients in France, PCT was monitored over four days, as part of the patients' standard of care and the change in PCT values were related to appropriateness of antibiotic therapy. Patients in the ICU who had bacteremia, community-acquired pneumonia, or ventilator-associated pneumonia

المنارة للاستشارات
www.manaraa.com

were prospectively enrolled in the study. The appropriateness of antibiotic therapy was determined by retrospective chart review to obtain antimicrobial susceptibility testing reports by an infectious disease expert who was blinded to the PCT values and patient outcomes. PCT values were also obtained from the retrospective chart review. The authors concluded that appropriate antibiotic therapy (the isolated pathogen(s) was (were) susceptible to at least one drug administered at the onset of sepsis according to the corresponding susceptibility testing report) was associated with a greater decrease in PCT values in the first 48 hours of antibiotic treatment (Table 3). The patient group that had appropriate antibiotic therapy had a greater decrease in mean PCT values between days two and three, -3.9 ng/mL, than the inappropriate antibiotic therapy group, +5.0 ng/mL ($p < 0.01$). The study also found that there was a greater rise in mean PCT values between days one and two for the inappropriate antibiotic therapy group, +5.2 ng/mL than for the appropriate antibiotic group, +1.7 ng/mL ($p = 0.20$). They noted, however, that their study was a single center study and that a prospective study would be needed to assess PCT and clinical findings as an indicator of the effectiveness of antibiotic therapy and the use of more effective therapies (Charles, 2009).

Table 3: Comparison of SPCTC Values in Appropriate and Inappropriate Antibiotic Therapy

	Antibiotic Therapy		
	Appropriate	**Inappropriate**	**p-value**
Number of subjects	135	45	
Average Day 1 PCT [ng/mL]	27.2	29.6	0.92
Average Day 2 PCT [ng/mL]	27.4	40.9	0.09
Average Day 3 PCT [ng/mL]	24.4	34.4	0.12
Average Day 4 PCT [ng/mL]	17.3	32.4	0.03

www.manaraa.com

Overall these studies and others (Bouadma et al., 2010; Tang et al., 2009) indicate that PCT can be used to decide if antibiotic therapy is required in the case of suspected bacterial infection. PCT may be useful in determining if antibiotic therapy is appropriate prior to the availability of antimicrobial susceptibility results.

Procalcitonin and Liver Disease

Initiation of antibiotic therapy as soon as possible is critical in all cases of bacterial infection and sepsis. However, in the ALF/ALI population this can be a double-edged sword. Many commonly used antibiotics are known to be metabolized by the liver. Examples include but are not limited to sulfa-related compounds, quinolones, amoxicillin, and flucloxacillin. Giving a drug that is metabolized by the liver to a patient with liver dysfunction can lead to drug toxicity. Some cases of ALF/ALI are caused by use of antibiotics. The reasons that antibiotics are associated with liver injury are not entirely understood (Murray, Hadzic, Wirth, Bassett, & Kelly, 2008). Therefore, giving antibiotics to this population of patients is not always the best course of action. A clear indication of need is of extreme importance in this situation. Therefore, a method of determining the need for antibiotic use that is faster than the standard culture methods is a worthwhile goal. Procalcitonin could prove to be highly useful for this group of patients, to either identify or exclude bacterial sepsis.

There are few published studies on the measurement of serum procalcitonin in patients with liver disease, and none specifically in patients with ALF or ALI. Previously published studies included populations with cirrhosis (Bota, Van Nuffelen, Zakariah, & Vincent, 2005; Connert, Stremmel, & Eising, 2003; Spahr, Morard, Hadengue, Vadas, &

المنارة للاستشارات
www.manaraa.com

Pugin, 2001; Viallon et al., 2000), liver metastasis of non-liver primary solid tumors (Matzaraki et al., 2007), and acute and chronic liver disease (Elefsiniotis, Skounakis et al., 2006). The results of the cirrhosis studies present some conflicting data. Viallon et al. (2000) enrolled 61 subjects: 21 with spontaneous bacterial peritonitis (SBP) and 40 with sterile ascitic fluid (SAF). The severity of liver disease was similar in both groups with the SBP patients having a mean Child-Pugh score of 11 and the SAF group having a Child-Pugh score of 10. The Child-Pugh score is a standard measure of the severity of chronic liver disease based on a three point evaluation of five features of cirrhosis: the presence/severity of ascites and encephalopathy and the concentrations of albumin, bilirubin, and INR. The maximum score is 15. The median PCT values from samples taken at study admission prior to initiation of antimicrobial therapy were significantly different between the two groups, with patients in the SBP group having a mean serum PCT = 10.10 ng/mL while that of patients in the SAF group was 0.09 ng/mL (p-value = 0.0001) (Viallon et al., 2000). The serum PCT measurements appear to be a marker of bacterial peritonitis in patients with liver disease.

Connert et al. (2003) enrolled 127 patients with cirrhosis. The subjects were divided into three groups based upon bacteriological and clinical findings. Group I had 36 patients with decompensated liver disease (ascites, encephalopathy, variceal hemorrhages are the classic features evidencing decompensation) with an infection. Group II had 64 patients with decompensated liver disease without an infection. Group III had 27 patients with non-decompensated liver disease and without infection. The mean PCT values for Groups II and III were lower (0.6 ng/mL and 0.4 ng/mL, respectively) than for Group I

المنارة للاستشارات
www.manaraa.com

(2.8 ng/mL) (Connert et al., 2003). Thus, serum PCT measurements appear to be a marker of infection in patients with cirrhosis.

Bota et al. (2005) studied 864 critically ill patients in the ICU, of which 79 were patients with cirrhosis. The cirrhotic patients were divided into three groups based upon severity of liver disease as measured by the Child-Pugh score. Table 4 illustrates that the serum PCT concentrations did not appear to vary with the severity of liver disease. The study did show a difference between infected and non-infected patient PCT values. Initial mean PCT values were approximately 1.25 ng/mL in infected cirrhotic patients, 2.0 ng/mL in infected non-cirrhotic patients, 0.5 ng/mL in non-infected cirrhotic, and 0.4 ng/mL in non-infected non-cirrhotic patients, but there were no significant differences in the PCT levels in relation to the severity of cirrhosis (Bota et al., 2005).

Table 4: SPCTC and Child-Pugh Score in Patients with Cirrhosis, With and Without Infection

	Controls	**Patients with Cirrhosis**						
		All	**Inf**	**NI**	**Inf**	**NI**	**Inf**	**NI**
# subjects	785	79	15	14	10	13	13	14
Child-Pugh score			5 - 6		7 - 9		10 - 15	
SPCTC [ng/mL] – initial	0.632	0.682	1.2	0.3	1.3	0.4	1.1	0.4

Inf = Infection; NI = No Infection

Spahr et al. (2001) measured serum PCT concentrations in 10 patients with cirrhosis and spontaneous bacterial peritonitis and in 10 patients with cirrhosis but without spontaneous bacterial peritonitis. The mean PCT values for 10 patients with SBP were 0.74 ng/mL vs. 0.2 ng/mL for 10 non-infected patients. The authors concluded that PCT was not a good indicator of infection in this group of patients possibly because the

المنارة للاستشارات
www.manaraa.com

absence of SIRS in the patients with SBP (Spahr et al., 2001). Therefore, serum PCT measurements may not be a marker of infection in patients with liver disease, if the infection is localized and the patient has not yet progressed to sepsis.

Matazaraki et al. (2007) studied the serum PCT concentrations in patients who had solid tumors that had metastasized to the liver. This study included 15 healthy control subjects (Group A), 21 patients with solid tumors that had not metastasized (Group B), 11 patients with liver metastasis (Group C), and 11 patients with generalized metastasis (Group D). The PCT values were 0.284 ng/mL for Group A, 0.327 ng/mL for Group B, 0.690 ng/mL for Group C, and 1.030 ng/mL for Group D. Table 5 shows the comparisons between the groups. PCT concentrations appear to increase with increasing degree of metastasis to levels that are similar to those found in patients with confirmed infection or sepsis. Further study will be required to determine if PCT can be used as a marker for sepsis and possibly as a marker of disease progression in this population of patients (Matzaraki et al., 2007).

Table 5: SPCTC in Patients with Liver Cancer

	Mean PCT (ng/mL)	**Comparison to Group C p-value**	**Comparison to Group D p-value**
Group A (control)	0.284	0.10	<0.001
Group B (solid tumor)	0.327	0.117	0.004
Group C (liver metastasis)	0.690		0.199
Group D (general metastasis)	1.030		

Elefsiniotis, Skounakis, et al. (2006) evaluated the differences in serum PCT concentrations between patients with acute and chronic liver disease. PCT was measured in 106 consecutively hospitalized patients with liver disease at the time of admission. The

المنارة للاستشارات
www.manaraa.com

etiology of liver disease included alcoholic hepatitis (Group A, n = 15), alcoholic cirrhosis without hepatitis or bacterial infection (Group B, n = 20), decompensated cirrhosis with proven bacterial infection (Group C, n = 16), uncomplicated viral hepatitis-cirrhosis (Group D, n = 42), and acute icteric viral hepatitis (Group E, n = 13). The mean SPCTCs were 0.40 ng/mL in Group A, 0.23 ng/mL in Group B, 9.80 ng/mL in Group C, 0.21 ng/mL in Group D, and 0.37 ng/mL in Group E. This study showed that while all groups without indication of infection had initial PCT values that were slightly higher than non-infected non-cirrhotic patients (LUMItest reference range PCT <0.3 ng/mL), PCT values from groups A, B, C, and E were significantly lower than the PCT values of the cirrhotic patients with infection (Group C) (Elefsiniotis et al., 2006).

Summary

All of the above studies, with the exception of the Spahr study, indicated that PCT can be used to presumptively identify a bacterial infection in patients with liver disease. However, none of the studies included patients with the most severe, acute liver disease, those with ALF or ALI. The Elefsiniotis study looked at some acutely ill liver disease patients some of whom might have met the diagnostic criteria of ALF or ALI, but none of the groups as a whole appeared to have met the diagnostic criteria. All of the above studies were small and none looked at how PCT relates to the severity of infection in patients with ALF or ALI. In addition, none of the studies explored whether or not SPCTCs change in response to successful treatment of bacterial infection in acute liver disease patients.

المنارة للاستشارات
www.manaraa.com

Significance of This Study

Procalcitonin appears to be a robust marker for bacterial infection. The above studies have shown that SPCTC is a good indicator of infection in the general population and is useful in monitoring antimicrobial therapy. However, it has not been extensively studied in the setting of acute liver injury or liver failure. The present proposed study investigated how SPCTCs vary in patients with ALF and ALI depending upon the severity of illness. Next, it will examine the utility of serum PCT concentrations as an indicator of bacterial infection in ALF and ALI patients. Lastly, the study investigated whether serum PCT values differ between ALF and ALI patients who are treated for bacterial infection and who achieve transplant free survival (alive at 21 days without transplant) and those treated for bacterial infection and who had a liver transplantation or died within the 21 day period of ALF or ALI study.

للاستشارات المنارة

www.manaraa.com

CHAPTER 3: METHODS

This chapter describes the research design, methodology, and analyses used to determine whether procalcitonin is effective as a biomarker of infection/sepsis in the ALF/ALI patient populations. The ALF and ALI databases, sample selection, and independent and dependent variables in each section of the study are described. Statistical methods used to analyze the data are described. Limitations of the study are discussed.

Research Design

This study was a retrospective, non-experimental study with three parts. A true experimental study was impractical due to the low incidence of ALF and ALI cases in the general population. Because the study used retrospective data and samples, the independent variable for this study, infection, could not be manipulated, fitting the description of non-experimental as described by Polit and Beck (Polit & Beck, 2008). Specific Aim 1 of the study examined the relationship between the severity of infection and SPCTC. Specific Aim 2 examined the relationship between the presence or absence of infection and SPCTC. Finally, Specific Aim 3 examined the differences in SPCTCs between infected ALF and ALI patients with transplant free survival (TFS - alive at discharge or 21 days) and infected ALF and ALI patients who died or received a liver transplant (DoT) within 21. The changes in the SPCTC values across the seven days of

المنارة للاستشارات
www.manaraa.com

the ALF/ALI study specimen collection were also compared between the TFS and DoT subjects.

Specific Aims, Hypotheses, and Study

There are three main aims for this study:

Specific Aim 1: To determine if 1) there are differences in SPCTCs between non-infected ALF and ALI patients and non-infected chronic liver disease patients, 2) between infected and non-infected ALF and ALI patients, and 3) between ALF and ALI patients and those of the general population with different severities of infection.

As noted by O'Grady, et al. (2008) and Harbarth, et al. (2001), SPCTCs can be used to denote the severity of infection in patients with definite or suspected bacterial infections. Can PCT be used in the ALF/ALI patient population to detect bacterial infections? Does liver disease affect the SPCTCs of liver disease patients with or without infection?

H_0: There is no difference in SPCTCs between non-infected ALF/ALI patients and non-infected chronic liver disease patients.

H_0: There is no difference in SPCTSs between non-infected patients with liver disease (chronic and ALF/ALI) and non-infected patients in the general population (i.e., SPCTC <0.1 ng/mL.

H_0: There is no difference in SPCTC between ALF/ALI patients with different degrees of severity of infection (i.e., Neg SIRS, SIRS, sepsis, severe sepsis, and septic shock).

المنارة للاستشارات
www.manaraa.com

These hypotheses were tested by measuring SPCTC in ALF/ALI patients with a documented history of infection, ALF/ALI without an infection, and chronic liver disease patients without an infection. The results were analyzed according to the presence or absence of infection and according to the severity of infection status based upon clinical signs and symptoms.

Specific Aim 2: Determine the utility of SPCTC as a screening biomarker for infection in ALF/ALI patients

Does a change in SPCTC predict the onset of bacterial infections/sepsis in the ALF/ALI patient population?

H_0: There is no difference in SPCTC in ALF/ALI patients between the first day a positive culture was collected and the three days prior to the positive culture.

By measuring SPCTC on the days prior to diagnosis of infection and on the day of infection, we determined if PCT will give an earlier indication of infection than clinical symptoms.

Specific Aim 3: Determine if SPCTC in ALF and ALI patients with bacterial infection on Day 1 of the ALF or ALI study is associated with patient outcomes.

Can changes in PCT be used to monitor progression or resolution of bacterial infection in the ALF/ALI patients?

H_0: There is no difference in SPCTC between ALF and ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.

المنارة للاستشارات
www.manaraa.com

H_0: There is no difference in changes in the serial SPCTCs between ALF/ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.

By measuring SPCTC in ALF and ALI patients who have known infections, we determined if there were differences in the SPCTC between patients who died or received a liver transplant and those with transplant free survival. In studies of SPCTC in subjects with pathologies other than liver disease and sepsis/bacterial infections, monitoring SPCTC has shown the ability to successfully decrease the length of antibiotic use (Bouadma et al., 2010; Kollef, 2010). We analyzed serial SPCTC in patients who were diagnosed with infection/sepsis on Day 1 of study and compared the results between those with transplant free survival and those who died or received a liver transplant. Changes in the serial values will also be analyzed to determine if there are differences between the two groups.

Databases

Samples and data were obtained from four liver disease sample repositories and databases maintained by the University of Texas Southwestern Medical Center (UTSW) (Table 6). Acute liver disease samples and data were obtained from the ALF and ALI databases and sample repositories. Chronic liver disease data and samples were obtained from the Liver Disease database and sample repository and from the Nucleic Acid, Serum, and Tissue Repository for the Study of Liver Diseases database and sample repository.

المنارة للاستشارات
www.manaraa.com

Table 6: Databases

Database	Collection Started	Description	Subjects Enrolled Prior to 10-1-10
Acute Liver Failure	Jan. 1998	A multi-center NIH supported study to collect clinical information, serum/plasma, DNA, and tissue samples from patients with ALF	1700
Acute Liver Injury	Oct. 2008	A multi-center NIH supported study to collect clinical information, serum/plasma, DNA, and tissue samples from patients with ALI	163
Liver Disease Database	Dec. 2005	An Investigator initiated study at UTSW to collect information and serum/plasma samples from subjects who may qualify for clinical trials or are at high risk for developing liver disease.	475
Nucleic Acid, Serum, and Tissue Repository for the Study of Liver Diseases Database	1996	Investigator initiated study at UTSW to collect data/samples to support translational research in liver diseases.	206

Acute Liver Failure Database

The U.S. Acute Liver Failure Study Group (USALFSG) was established in 1998 with pilot funding from the FDA and supported by the National Institute of Health (NIH) continuously since that time. The group proposed two aims: to prospectively collect detailed clinical information, serum, DNA, and tissue samples from patients with ALF and to develop controlled trials of innovative therapies for ALF (Lee et al., 2008; Lee & Seremba, 2008). The USALFSG is currently composed of 13 clinical sites, reduced from a maximum of 23 clinical sites prior to 2010. From January 1998 to October 2010, 1700 adult ALF patients have been prospectively enrolled in the study. Inclusion criteria

www.manaraa.com

defining ALF are the presence of coagulopathy (PT > 15 seconds or INR $\geq$1.5) and any grade of hepatic encelphalopathy (HE) that occurs within 26 weeks of the first onset of symptoms. The patients may not have any previously diagnosed liver disease, with the exception of chronic hepatitis B or Wilson's disease as defined earlier (Lee et al., 2008). Due to the altered mental status of the patients caused by HE, informed consent must be obtained from the next of kin to be enrolled in the ALF study.

Patients are enrolled into the ALF study for a maximum of 21 days with two annual follow-up visits. Clinical and demographic information, medical history, and medication history are obtained from the medical record for days 1 – 7 and 21 (or day of discharge, transplant, or death) and at each follow-up visit. The information is recorded on the study case report form (CRF). If required, information may be requested from the patient's primary care physician, once a signed medical records release form is obtain. All information is entered into the USALFSG database.

Blood samples are collected on days 1 – 7, unless the patient is discharged, dies, or is transplanted. The blood samples are centrifuged to remove the cells and 500 µL aliquots of serum or plasma are frozen at -20° C or colder in small cryo vials. Samples are stored at the collecting facility until sent to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sample repository. The NIDDK repository stores the samples at -70° C.

Acute Liver Injury Database

The Acute Liver Injury Study is an extension of the ALF study in which patients present to the hospital with acute liver disease but do not exhibit any signs of hepatic

المنارة للاستشارات
www.manaraa.com

encephalopathy at the time of admission. If encephalopathy occurs during the course of the study, the patient may be consented and enrolled in the ALF study. In this case, the data would then be collected using the ALF protocol. The purpose of the ALI study, like the ALF study, is to collect clinical information, serum, DNA, and tissue samples from patients with ALI, and to develop controlled trials of innovative therapies for ALI. The ALI subjects are prospectively enrolled into the study at the same clinical sites that are enrolling ALF patients. The data and sample collection plan is similar to that described for ALF. Data and blood samples are collected on days 1 – 7 (or until discharge, death, transplant, or transfer to the ALF study). Follow-up data is also collected at day 21, six weeks, and 12 weeks if possible. The ALI sub-study was started in October 2008 and has enrolled 163 subjects through September 2010.

Although the diagnostic criteria for acute liver injury are the same as for acute liver failure only without HE, the inclusion criteria for the ALI database differ slightly from those of ALF. The inclusion criteria for ALI are: serum ALT greater than or equal to 10 times the upper limit of normal, total serum bilirubin greater than or equal to 3.0 mg/dL, coagulopathy (PT greater than 20 seconds or INR greater than 2.0), acute hepatic illness of less than 26 weeks and no evidence of encephalopathy. Patient with acetaminophen etiology should have hepatic illness of less than two weeks duration.

Liver Disease Database

The primary purpose of the Liver Disease database (LDD) is to allow for contact between study personnel at the University of Texas Southwestern Medical Center at Dallas (UTSW) Clinical Center for Liver Disease (CCLD) and patients who have liver

المنارة للاستشارات

www.manaraa.com

disease and may wish to qualify for clinical trials or who are considered at high risk of developing liver disease (subjects with high risk behavior and spouses of patients with hepatitis). The secondary purpose of the database/sample repository is to provide serum samples for various liver disease research projects, and to characterize subjects based on their specific disease etiology such as viral hepatitis genotype. While the study enrolls subjects with all types of liver disease and some without apparent liver disease, it is composed primarily of subjects with viral hepatitis, predominantly hepatitis C. The study received initial IRB approval in December 2005, and has prospectively enrolled 475 subjects as of Oct. 2010.

Once a patient is identified as a possible study subject and informed consent is properly executed, a short questionnaire is filled out. The questionnaire is used to collect demographic information including contact information and liver disease etiology. If the subject consents, a blood sample is collected so that serum and plasma can be stored for later use to categorize the subject or for research protocols involving liver disease. The subject may elect to not have a blood specimen collected at the time of enrollment but may be asked to have one collected at a later time if they are interested in a potential treatment study and wish to be pre-screened for the trial. Remnant samples are stored for future testing. The blood samples are centrifuged to remove the cells and 500 μL aliquots of serum or plasma are frozen at -20° C or colder in small cryo vials. Samples are stored at UTSW Liver Research Laboratory at -70° C.

المنارة للاستشارات

www.manaraa.com

Nucleic Acid, Serum, and Tissue Repository for the Study of Liver Diseases Database

The primary purpose of the Nucleic Acid, Serum, and Tissue Repository for the Study of Liver Diseases (NSTLD) study is to gain a better understanding of possible causes of the development and progression of liver diseases in order to develop new treatments. The data/sample repository provides support for translational research in liver diseases within the UTSW Division of Digestive and Liver Diseases. While the study enrolls subjects with all types of liver disease and controls, the database is composed primarily of subjects with primary biliary cirrhosis. The study received initial IRB approval in 1996, and has prospectively enrolled 206 subjects as of Oct. 2010.

Once a patient is identified as a possible study subject and informed consent is properly executed, a short questionnaire is filled out. The questionnaire is used to collect demographic information including contact information and liver disease etiology. Blood and tissue samples are collected as part of this study. The blood samples (for obtaining serum or plasma collection) are centrifuged to remove the cells and 1000 μL aliquots of serum or plasma are frozen at -70° C or colder in small cryo vials. Samples are stored at the UTSW Department of Digestive and Liver Diseases Laboratory at -70° C.

Sampling

This study has three components. Each part of the study is related to one of the three specific aims. Sample populations are described for each group.

Specific Aim One

The first step requires the identification of the patient samples used for this section of the study. As described above, a patient can be classified as having SIRS,

المنارة للاستشارات
www.manaraa.com

sepsis, and severe sepsis based upon their clinical biodata. An algorithm (see Appendix A) based upon the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference definitions of SIRS, sepsis, severe sepsis, and septic shock was used to determine which classification each subject fit into during each of the first seven days of the study for all subjects in both the ALF and ALI databases. The algorithm was based upon the available data contained in the databases.

Subjects with SIRS, by definition, have two or more of the following signs or symptoms: body temperature $>38^{o}$ C or $<36^{o}$ C, heart rate >90 bpm, respiration rate >20 breaths/min, PCO_2 level <32 mm Hg, WBC >12,000 or <4,000, or bands (immature granulocytic white blood cells) >10%. The ALF and ALI databases do not collect data for heart rate and respiration for all seven days and does not routinely collect data on bands. These parameters were not used to identify patients with SIRS but were used to identify patients without SIRS.

Sepsis is defined as the presence of SIRS plus a documented infection or the presence of at least one of the following signs or symptoms: edema or positive fluid balance, glucose >120 mg/dL in the absence of diabetes, elevated CRP or PCT values, a SVO2 >70%, or a cardiac index of >3.5 L/min/M^2. In the case of the ALF and ALI databases, none of the signs and symptoms data were collected on all days of the study. Therefore, a classification of sepsis was made only when a patient had a SIRS classification plus a confirmed infection (positive culture). Positive bacterial cultures are considered the gold standard for infection, although this strict criterion may also miss certain infections where a positive culture is not obtained for a variety of technical

المنارة للاستشارات
www.manaraa.com

reasons. The presence of yeast and fungal infections was captured in the infection data but was excluded from the analysis because a positive bacterial culture was used as the indication of an infection. There is published evidence that yeast and fungal infections cause an increase in SPCTC but at a lower level than those of bacterial infections (Dornbusch et al., 2005; Martini et al., 2010; Nakamura, Wada, Takeda, & Nobori, 2009).

Severe sepsis is defined as organ failure in the presence of sepsis. Because a diagnosis of organ failure (i.e. liver failure) has already been made by definition in the ALF population, all ALF patients would automatically be classified as severe sepsis. For the purposes of this study, subjects were not categorized into the severe sepsis group if **only** liver failure was noted. A subject was classified into the severe sepsis category if any additional type of organ failure other than liver failure was noted. Table 7 lists the various biodata parameters used to denote organ failure and the type of organ failure suggested by the parameter. The parameters acute oliguria, prolonged aPTT, and diminished blood pressure as noted in the previous discussion, were not used as information relevant to these parameters was not consistently collected for all days of the study.

Septic shock is defined as the presence of severe persistent hypotension despite adequate volume resuscitation (Levy et al., 2003) in the presence of sepsis. Because the majority of the patients in this group were in the intensive care unit of the hospital, an assumption of adequate fluid resuscitation was made (treatment for hypotension). A MAP of <60 mmHg while on pressor therapy was used as an indicator of persistent

المنارة للاستشارات
www.manaraa.com

Table 7: Severe Sepsis Signs and Symptoms

Severe Sepsis	
Parameter	**Organ system**
Arterial hypoxemia PaO2/FiO2 ratio <300 torr	Respiratory
Creatinine >0.5mg/dL increase above baseline	Kidney
Coagulation abnormalities INR >1.5	Coagulation - blood
Thrombocytopenia platelet count <100,000	Coagulation - blood
Hyperbilirubinemia total bilirubin >4 mg/dL	Liver
Hyperlactatemia >1 mmol/L	Blood
MAP <70 mmHg	Respiratory
Altered mental status Coma Grade	Central nervous system

hypotension. Blood pressure parameters other than MAP were not utilized because information on these parameters was not collected on all days of the studies.

Non-infected/non-SIRS ALF/ALI (Neg SIRS) samples were selected from patients who met no more than one of the SIRS criteria. These samples were selected from Day 1 of the study so that all parameters (WBC, pulse, respiration, temperature, and pCO_2) were available identify patients in this category. Samples with missing SIRS criteria data were excluded.

The data were organized into a Microsoft Excel 2007 spreadsheet (Microsoft, Redmond, WA) and condition statements (e.g., IF, AND, OR, etc.) were used to determine the status of each ALF and ALI patient on each day of study enrollment. The following guidelines were used to indicate a specific category:

المنارة للاستشارات
www.manaraa.com

- Neg SIRS – if less than two of the SIRS parameters were positive
- SIRS – if any two of the SIRS parameters were positive
- Sepsis – presence of a confirmed bacterial infection
- Severe sepsis – the presence of organ failure other than liver
- Septic shock – extreme hypotension with pressor therapy

An individual subject may multiple categories during the course of the seven days of the study however, that subject was not used for more than one category.

Because the ALF and ALI studies are non-treatment studies that were not planned to specifically study the SIRS continuum and infection, specific data were not available for each patient on all days of the study. Some of the biodata parameters noted in the SIRS categories are not routinely collected by the ALF and ALI studies and the attending physician may not have required other parameters. For the 1863 patients enrolled in the ALF and ALI studies, there are 12,492 days of demographic and biodata information available. However, only 3.5% of all days that all patients were enrolled in the studies had complete data for all biodata parameters. Of those with complete biodata, only 80 days had a documented infection. The sampling plan for Specific Aim 1 described below calls for 90 samples with a documented infection. Therefore, to maximize the availability of samples, missing data were treated as a null value (i.e., if a lab value was missing, it was considered to be negative for that parameter) for the purposes of categorizing the samples. A biodata parameter had to be present and fit the criteria definition to be considered positive.

المنارة للاستشارات
www.manaraa.com

A power analysis was performed to determine an appropriate sample size. First, we estimated the effects of sample size using results from the literature (Giamarellos-Bourboulis et al., 2002a) and log-transformed the means and standard deviations ($\log_{10}$) due to the large differences in variances for each group. A sample size estimate using a one-way ANOVA design with five levels (not infected, SIRS, sepsis, severe sepsis, and septic shock) resulted in an effect size of 0.65, a large effect by Cohen's standards, (Cohen, 1977). Using this effect size estimate conservatively, a power analysis was conducted for a factorial design with two between factors at three (etiology: APAP, viral, and other) and 5 (infection categories) levels. Different infection groups were assumed to have a medium effect (0.30), different etiologies were assumed to have a small effect on the PCT values (0.15), and the interaction (etiology by infection) was assumed to have a small effect (0.15). A minimum of 10 observations in each of the 15 cells (3 x 5) for a total of 150 observations will achieve the following levels of power. An 83.8% power for the comparisons of the infection groups, 35.0% power for the comparisons of the etiologies, and 19.2% power for the interaction between etiologies and infection group will result using an F test assuming an alpha of 0.05.

After determining the SIRS status of each ALF and ALI subject on each day of study enrollment, samples were selected based upon the following schema described in Table 8. The ALF/ALI project manager was given a list of possible samples for each category. The project manager selected the appropriate number of samples from the list of possible samples for each category based upon sample availability. If a subject was in the same category for more than one day, only one day was used for that subject. The day

المنارة للاستشارات
www.manaraa.com

Table 8: Categories and Numbers of Subjects Desired for Specific Aim One

Classification	Etiology of Liver Disease			Total Classification
	Acetaminophen	Viral hepatitis	Other Etiologies	
Chronic/ non-ALF, not infected	na	10	10	20
Neg SIRS, not infected	10	10	10	30
SIRS, not infected	10	10	10	30
Sepsis	10	10	10	30
Sever sepsis	10	10	10	30
Septic shock	10	10	10	30
Total etiology	50	60	60	170

tested was the first day the subject fell into the category for which a serum sample was available. Subjects from the Liver Disease and NSTLD databases were outpatients and were considered to have less severe liver disease than the hospitalized ALF and ALI subjects, negative for SIRS criteria, and classified as not infected. The chronic/non-ALF/non-ALI subject category does not have an acetaminophen etiology since liver disease caused by an overdose (either accidental or intentional) of acetaminophen is acute, not chronic in nature.

The subjects were distributed between the different etiologies so that all types of acute liver failure/injury were represented in the study. Etiologies were examined to determine if they resulted in differences in SPCTC results. The SPCTC results from the chronic group and the non-infected groups were compared to the published SPCTC reference range for non-infected individuals (SPCTC <0.1 ng/mL) to determine if there is a different reference range for patients with liver disease.

المنارة للاستشارات

www.manaraa.com

Specific Aim Two

Forty-five subjects who had a positive bacterial culture identified on days four, five, six, or seven in either ALF or ALI study were selected for SPCTC measurement. A list of potential subjects was generated. The AFL/ALI project manager selected the subjects based upon sample availability. Samples were obtained from the day of the positive bacterial culture and from each of three preceding days and analyzed for SPCTC.

Specific Aim Three

Subjects who had a positive bacterial culture on Day 1 of the study were categorized based upon their end of ALF or ALI study outcome. The subjects in this section of the study were categorized as having either transplant free survival (TFS) or death or liver transplant (DoT) by Day 21. If patients were placed on the liver transplant wait list, this factor alone was not considered a bad outcome because a patient might have improved and removed from the list. United Network for Organ Sharing (UNOS) status one criterion is limited to ALF patients (sudden and severe onset of liver failure) and states that a patient has a high likelihood of dying within seven days ("Questions and answers for transplant candidates", 2008). If a patient was placed on the liver transplant wait list and remained on the wait list through day 21, this patient was excluded from either category. A list of subjects that fit each group was generated. The ALF/ALI biodata manager selected 30 subjects from each group (TFS or DoT) based upon which samples had the most available volume. One blood sample from each of the seven sampling days each subject was enrolled in the ALF or ALI studies was requested (if available).

المنارة للاستشارات
www.manaraa.com

Testing

Specimen Procurement and Retrieval

A list of samples from each section of the study was given to the USALFSG coordinator in charge of the sample repository to determine the availability of samples. Once sample availability was confirmed, a list of the requested samples was sent to the NIDDK. The NIDDK retrieved the requested samples and shipped them to study personnel at Siemens Healthcare Diagnostics in Tarrytown, NY for testing. In some cases, if a specimen was requested for more than one specific aim, the SPCTC results from one specimen were utilized for all specific aims. Frozen samples were shipped on dry ice using a commercial carrier. Upon receipt by study personnel at UTSW, the samples were stored at -70° C until testing. Samples from the LDD and NSTLD sample repositories at UTSW were also shipped on dry ice to Tarrytown, NY by commercial courier for testing.

ADVIA Centaur Immunoassay Analyzer Testing

The ADVIA Centaur system uses a Master Curve and two-point calibration to calibrate quantitative assays such as the PCT assay. The master curve is established as part of the manufacturing process. Full standard curves are performed on a lot number of PCT reagent on multiple instruments at multiple concentration levels. The RLUs at each level is determined. The concentrations of the standards and the RLUs at each concentration are used to generate a master curve for that lot. The PCT assay is a sandwich assay that produces a calibration curve that has a positive slope. To minimize instrument-to-instrument variability, reagent age, and environmental factors, a two-point

المنارة للاستشارات
www.manaraa.com

calibration is performed when a new lot of reagent is installed on the instrument, at specified intervals (35 days for PCT), and as needed (example: out of range control values or major maintenance procedures). Replicates of two defined calibrators are measured on the instrument using the assay sequence described below. If the mean RLU values for the calibrators meet defined validity criteria, the system compares the calibrators to the Master Curve for the lot of PCT reagent and determines a system-specific formula for the lot of PCT reagents. The system then uses that lot/system-specific formula to determine the PCT values of subsequently run controls and patient samples ("Advia centaur reference manual", 2003).

The ADVIA Centaur BRAHMS PCT ("Advia centaur systems - procalcitonin (PCT)", 2010) assay is a sandwich immunoassay composed of solid phase, lite reagent, and ancillary reagents. The solid phase reagent contains a monoclonal mouse antibody to fluorescein covalently linked to paramagnetic particles. The lite reagent contains a monoclonal mouse antibody to procalcitonin that is labeled with acridinium ester. The ancillary reagent contains a monoclonal mouse antibody to procalcitonin labeled with fluorescein (Figure 2).

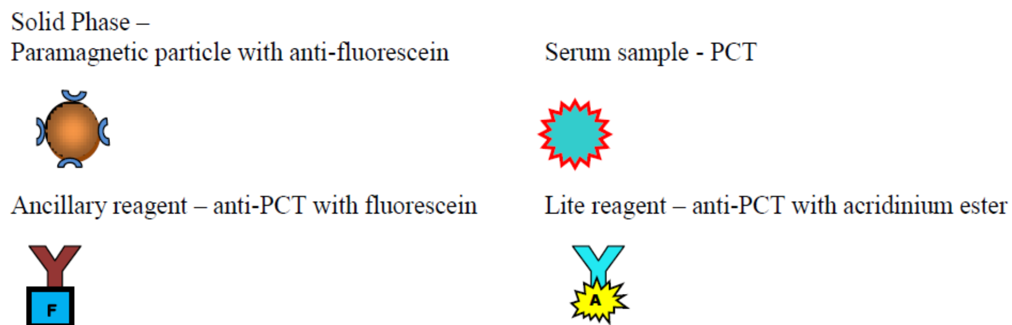

Figure 2: Siemens PCT Reagent Components

المنارة للاستشارات

www.manaraa.com

Serum samples were thawed, mixed and placed in bar-coded sample holders and racks that were then placed on the Centaur for analysis. The ADVIA Centaur is a random access analyzer that can produce 270 PCT results in approximately two hours. Samples were tested in three groups (based upon which specific aim the sample was selected for) over the course of four days. Quality control samples (ADVIA Centaur BRAHMS PCT quality control samples 1 and 2) were run in triplicate at the beginning and end of testing on each day of testing. The first set of samples tested were those for Specific Aim 1. The second and third sets of samples tested for were for Specific Aims 2 and 3, respectively. Samples requiring a dilution, a result above the analytical range or due to low sample volume, were tested on the fourth day using a manual dilution. Results were calculated from the reported result by multiplying by the appropriate dilution factor.

The ADVIA Centaur system automatically processed samples to be tested for PCT in the following sequence:

1. Combines 100 μL sample and 45 μL ancillary reagent incubates the mixture for 5.75 minutes at 37^0 C (Figure 3)

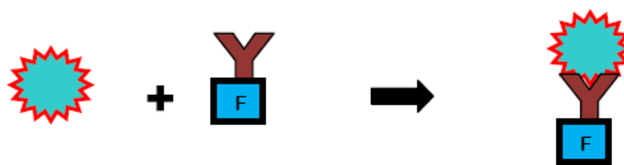

Figure 3: Sample + Ancillary Reagent

2. Adds 100 μL of solid phase and 50 μL of lite reagent to the mixture and incubates for 18 minutes at 37° C (Figure 4)

www.manaraa.com

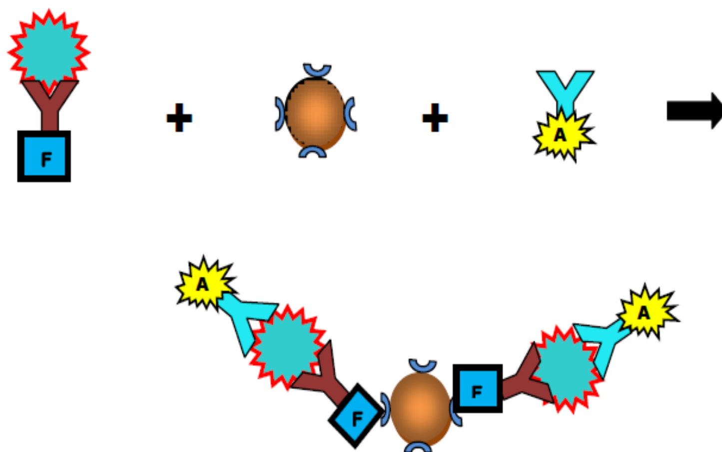

Figure 4: Add Solid Phase and Lite Reagent

3. Aspirates the unbound reagent, holding the solid phase in place in the reaction cuvette by means of a magnet
4. Washes the solid phase with Wash 1 solution (a phosphate buffered saline with sodium azide (<0.1%) and surfactant)
5. Aspirates the Wash 1 solution while holding the solid phase in the reaction cuvette by means of a magnet.
6. Adds 300 µL of Acid Reagent to start a chemiluminescent reaction with the acridinium ester.
7. Moves the reaction cuvette into the luminometer and adds 300 µL Base reagent completing the chemiluminescent reaction producing a flash of light.
8. The luminometer measures the intensity of light produced by the chemiluminescent reaction. The light is measured in relative light units (RLUs)

المنارة للاستشارات
www.manaraa.com

The amount of RLUs detected by the system is directly proportional to the amount of PCT present in the patient sample ("Advia centaur immunoassay system reference manual", 2003). The measuring range is 0.02 to 75 ng/mL.

Because of regulations restricting use of reagents that have been submitted to the FDA but have not been approved for use, testing was performed at Siemens Healthcare Diagnostics in Tarrytown, NY. A single ADVIA Centaur was made available for use during the four days required for testing. Siemens Healthcare Diagnostics, Tarrytown, NY, provided all reagents required for testing.

The assigned analyzer was calibrated using a 2-point Master Curve calibration. The master curve and calibrator values were entered into the Centaur computer system and low and high calibrators with known values were assayed in triplicate as required for calibration of a new lot of reagent. The average observed RLU values from the calibrators were compared to the Master Curve and a system-specific formula was generated. Patient and quality control samples were assayed and the observed RLU values were adjusted using the generated system-specific formula to obtain adjusted RLUs that were then compared to the Master Curve to obtain the final PCT concentrations. Samples with values greater than 75 ng/mL were manually diluted using Multi-Dil 1 ancillary reagent and the diluted samples were re-assayed as described above.

Statistical Analysis

Laboratory data from the databases were entered into Microsoft Excel 2007 spreadsheets. These data were merged with the procalcitonin data and analyzed using

المنارة للاستشارات

www.manaraa.com

SPSS 19.0 (SPSS Inc., Chicago, IL, 2010) and SAS 9.2 (SAS Institute Inc., Cary, NC, 2008).

Descriptive statistics are included with the analysis of all sample data for all groups. In the study, Specific Aim 1 addressed whether SPCTC (the dependent variable) can be used to detect bacterial infections in patients with ALF and ALI. Subjects were categorized by ALF etiology and infection with the level of infection based upon SIRS/sepsis criteria (the independent variables). A Kruskal-Wallis test with post hoc testing (Dunn Method) for non-parametric parameters was used to analyze the data to determine if SPCTC can be used to detect infection, if there is a difference between SPCTC in patients with chronic liver disease and ALF and ALI, and indicate the severity of infection in ALF and ALI patients. The chronic group and the non-infected groups (Neg SIRS and SIRS) means were compared to the reference range of the PCT assay. An expected value of <0.1 ng/mL was calculated from PCT values from a population of 456 normal subjects. The 95% confidence interval for the mean concentration was 0.023 to 0.028 ng/mL ("Advia centaur and advia centaur XP systems - procalcitonin (PCT)", 2010).

A Chi-square test was used as an alternative method for analyzing the Specific Aim 1 hypothesis. Use of chi-square required re-categorizing the samples into infection and non-infection groups. SPCTC results were dichotomized based upon a cut-off value indicative of infection. ROC analysis was used to determine the optimal cutoff value that separates the specimens from the infected and non-infected subjects. The optimal cut-off

المنارة للاستشارات
www.manaraa.com

value was defined as the point on the curve where the perpendicular distance from (and above) the 45° line of equality is at maximum (Riffenburgh, 2006).

Specific Aim 2 addresses the use of SPCTC as a screening marker for bacterial infection in ALF and ALI patients by determining the SPCTC (the dependent variable) on the day of identified bacterial infection and comparing it to the SPCTC for the three days prior to infection. The presence or absence of infection is the independent variable for this specific aim. The day of identified infection is positive, while the previous three days are assumed to be negative. Data pertaining to Specific Aim 2 were analyzed using a Friedman test for non-parametric related samples to determine if SPCTC can be used as a screening biomarker in ALF and ALI subjects who develop a bacterial infection.

Specific Aim 3 addresses the use of SPCTC to predict outcomes in ALF and ALI patients with a bacterial infection. Subjects were categorized as having transplant free survival or death or transplant outcomes (the independent variable). SPCTC (the dependent variable) values for each day of the seven day study period were determined. Data for Specific Aim 3 were analyzed using a mixed models analysis of covariance. The following variables were explored to determine their significance in the model: visit day, outcome (TFS or DoT), diagnosis (APAP vs. All Other Etiologies), severity of illness (sepsis, severe sepsis, and septic shock), antibiotic therapy (prescribed or not prescribed), antibiotic prophylaxis (prescribed or not prescribed), antibiotic use (prophylaxis and/or therapy - prescribed or not prescribed), and coma grade (1 and 2 (mild) vs. 3 and 4 (severe)). The results of the analyses were used to determine if mean SPCTC levels were different when accounting for features related to bacterial infection in ALF patients.

المنارة للاستشارات
www.manaraa.com

CHAPTER FOUR: RESULTS

This chapter describes the results from the statistical analysis of the data and explores the relationship between SPCTC and severity of illness, determines if SPCTC can detect infection, and determines if SPCTC was related to the final outcome in ALF and ALI patients. A brief summary of statistics related to the ADVIA Centaur BRAHMS assay will also be presented. For each specific aim, a description of how the samples were selected will be presented along with descriptive statistics for each group of samples. This will be followed by an overview of the results. Finally, detailed analyses for each hypothesis will be presented. The statistical analyses and graphics prepared for this study were performed using SPSS 19, SAS 9.2, Microsoft Excel 2007, and SigmaPlot 12.0 (Systat Software, Inc. San Jose, CA).

Institutional Review Board (IRB) approval was obtained from the University of Texas Southwestern Medical Center (IRB#STU092010-126) and Virginia Commonwealth University (IRB#HM13517). Study subjects were patients who had previously consented to enrollment into one or more of the following studies: ALF, ALI, LDD, or NSTLD.

Assay Statistics

ADVIA Centaur BRAHMS PCT assay is sandwich immunoassay developed for use with the ADVIA Centaur analyzer. The PCT assay was developed to aid in the

المنارة للاستشارات
www.manaraa.com

detection and monitoring of infection and sepsis in patients suspected of having a bacterial infection. ADVIA Centaur BRAHMS PCT low and high controls were run in triplicate each day after the initial calibration at the beginning of the sample set (morning) and at the end of the set (afternoon) on testing days one, two, and three. The controls were run only in the morning on day four as the run was completed within an hour. All control values fell within the prescribed ranges. The control values were used to calculate within-run and between-run means, standard deviations, and coefficient of variations (Table 9). Overall, the assay performed to the manufacturer's specifications. However, the CVs for this study were less than the CVs previously reported for the ADVIA Centaur BRAHMS PCT and the KRYPTOR PCT (between-run: 3.6% to 8.6% and within-run: 1.1% to 6.1%) (Aso, et al., 2009) .

Table 9: PCT Assay Imprecision

Control	Assigned Range	All Runs		With-in Run CV		Between-Run CV
		Mean	SD	Lowest	Highest	
Low (ng/mL)	0.221 – 0.663	0.40	0.014	0.67	2.93	3.41
High (ng/mL)	7.30 – 13.7	10.06	2.19	0.48	1.26	2.19

.

Specific Aim One

The purpose of Specific Aim 1 was to measure SPCTCs in infected and non-infected ALF and ALI patients to determine whether values observed were the same or different from the PCT ranges based upon the severity of infection noted in previous studies in the general population.

المنارة للاستشارات

www.manaraa.com

Sample Selection

Data from 1863 ALF and ALI cases (1829 patients – 34 were initially enrolled in the ALI study, subsequently developed HE, and were enrolled into the ALF study) were entered into an Excel 2007 spreadsheet. The clinical biodata on each available day for each subject was examined and sorted using an algorithm (see appendix A) to classify each patient-day as representing either SIRS, sepsis, severe sepsis, or septic shock. Data from 12,492 patient-days were sorted into the above categories. Because heart rate and respiration were collected only on Day 1, only biodata from Day 1 were analyzed for the Neg SIRS category. Table 10 presents the total number of patient-days identified for each severity category. The project manager randomly selected samples for each etiology for all severity categories based upon availability of serum samples in the sample repository. Table 11 presents the actual number of patient-days in each category and etiology.

Table 10: Category and Sample Availability of ALF and ALI subjects

Category	**Description**	**Number Patient Days Available**
Neg SIRS (Day 1)	Less than 2 of SIRS values positive	628
SIRS (Days 1 – 7)	2 or more SIRS values positive	1407
Sepsis (Days 1 – 7)	SIRS with a confirmed infection and only liver dysfunction	39 (from 29 patients)
Severe Sepsis (Days 1 – 7)	SIRS with a confirmed infection and multiple organ dysfunction	348
Septic Shock (Days 1 – 7)	SIRS with a confirmed infection and a MAP < 60	31 (from 25 subjects)

المنارة للاستشارات
www.manaraa.com

Table 11: Number of Subjects Tested for PCT Based Upon Severity Category and ALF Etiology

	Etiology of Liver Disease			
Classification	**Acetaminophen**	**Viral hepatitis**	**Other Etiologies**	**Total Classification**
Chronic/ non-ALF, not infected	na	10	10	20
Neg SIRS, not infected	10	10	10	30
SIRS	10	10	9	29
Sepsis	6	1	6	13
Sever sepsis	10	6	11	27
Septic shock	5	1	10	16
Total etiology	41	38	56	135

The sepsis group and the septic shock group had fewer available samples than the other categories. There are multiple possible reasons for this. The ALF patient population were critically ill. Samples and data were collected only once every 24 hours for the ALF and ALI studies and the patients' conditions could change within hours. In some cases, samples were either not collected on a given day or the supply of aliquots had been depleted. Single organ dysfunction (i.e., liver only) is extremely rare in this population. The same is true of septic shock. The septic shock patients are at the most severely ill end of the spectrum of illness. It is possible that more subjects may have actually fit into this category, but died or underwent liver transplantation before the next day's sample was collected. The apparent low number of viral hepatitis subjects in three of the categories reflects the small number of viral hepatitis cases (approximately 12%) among both ALF and ALI registries, while APAP accounts for approximately 50%. All other etiologies account for approximately 38%.

المنارة للاستشارات
www.manaraa.com

Available demographic and biodata information were obtained from the LLD and NSTLD databases for patients with chronic liver disease. In the case of the LLD database, information was limited to demographic information. The NSTLD database did have demographic information and biodata, but the amount of biodata was not as extensive as the biodata collected in the ALF or ALI databases. Because the NSTLD subjects were ambulatory (outpatients), it was assumed that the likelihood of them having an infection was extremely small and were classified as not infected.

Demographic Information

Differences in demographic information, biodata, and laboratory values among the severity categories were determined using one-way ANOVA for determining differences between group means or a 2-sided Fisher's Exact test for determining differences between group percentages at a statistical significance level of 0.05. A summary of the demographic information for each infection group is described in Table 12. There were significant differences in the ages (p-value = 0.030) and gender (p-value = 0.029), but not in race (p-value = 0.479) of the subjects among the infection groups. However, there was a higher percentage of females and caucasians than males and other races in the study over all.

There was considerably more variation in the biodata and laboratory data among the categories (Table 13). Pulse and respiration were significantly different between the categories as expected since they reflect severity of illness (p-value = 0.001 for each) while temperature and MAP were not. Note: The Septic Shock MAP was not included in

المنارة للاستشارات
www.manaraa.com

Table 12: Demographic Data for Patients Based Upon Severity of Illness Classifications

Mean (± SD) or Number (%)	**Chronic n = 20**	**Neg SIRS n = 30**	**SIRS n = 29**	**Sepsis n = 13**	**Severe Sepsis n = 27**	**Septic Shock n = 16**	**p-value**
Age (years)	53.8 (±9.4)	47.2 (±16.0)	40.5 (±15.8)	42.9 (±15.8)	40.7 (±12.5)	45.4 (±17.2)	0.030
Gender							
Female	10 (50)	15 (50)	21 (72.4)	12 (92.3)	21 (77.8)	12 (75)	0.029
Ethnicity*							
Hispanic		5 (16.7)	5 (17.2)	2 (15.4)	3 (11.1)	1 (6.7)	
Race*							
White	14 (77.8)	21 (66.7)	23 (79.3)	9 (69.2)	24 (88.9)	14 (87.5)	0.479
African American	1 (5.6)	5 (16.7)	5 (17.2)	1 (7.7)	3 (7.7)	1 (6.2)	
Asian	2 (11.1)	2 (6.7)	1 (3.4)	2 (15.4)		1 (6.2)	
Hawaiian		1 (3)					
Other	1 (5.6)	1 (3)		1 (7.7)			
Diagnosis							
APAP		10 (33.3)	10 (34.5)	6 (46.2)	10 (37.0)	5 (31.2)	
Viral Hepatitis	10 (50)	10 (33.3)	10 (34.5)	6 (46.2)	6 (22.2)	1 (6.2)	
Other	10 (50)	10 (33.3)	9 (31.0)	1 (7.7)	11 (40.7)	10 (62.5)	
Outcome (TFS)							
APAP		6 (20)	5 (17.2)	5 (38.5)	7 (25.9)	1 (6.2)	
Viral Hepatitis		4 (13.3)	2 (6.9)		2 (7.4)		
Other		1 (3.3)	4 (13.8)	2 (15.4)	3 (11.1)	1 (6.2)	
Coma Grade							
Not Reported / 0	20 (100)		4 (13.8)	1 (7.7)	2 (7.4)		
1		11 (36.7)	1 (3.4)	3 (23.1)	1 (3.7)		
2		11 (36.7)	8 (27.6)	1 (7.7)			
3		4 (13.3)	5 (17.2)	2 (15.4)	9 (33.3)	1 (6.2)	
4		4 (13.3)	11 (37.9)	6 (46.2)	15 (55.6)	15 (93.8)	
Culture Types							
Blood – 1				1	5	5	
Tracheal – 2				6	6	4	
Urine – 3				2	3	3	
Wound – 4				1	2	1	
Catheter – 5					1		
Multiple w Blood – 7				2	8	2	
Multiple wo Blood – 8				1	2	1	
Antibiotics Used		19 (63.3)	23 (79.3)	11 (84.6)	22 (81.5)	13 (81.2)	0.471
Prophylaxis		15 (50)	17 (58.6)	5 (38.5)	14 (51.9)	10 (62.5)	0.718
Therapy		7 (23)	12 (41.4)	7 (53.8)	15 (55.6)	7 (43.8)	0.122
Age of Sample (years)	8.4 (± 5.6)	6.6 (± 3.3)	6.8 (± 3.0)	7.2 (± 3.1)	6.3 (± 3.2)	6.2 (± 2.9)	0.380
After Hospital Admission (days)		3.9 (± 2.7)	8.6 (± 10.3)	5.8 (± 3.4)	8.2 (± 5.5)	7.2 (± 6.2)	0.002

*Ethnicity: O were reported in the Chronic group; 15 of 16 were reported in the Septic Shock group
Race: 18 of 20 reported in the Chronic group / p-value reported for white vs. all other races

www.manaraa.com

Table 13: Biodata and Laboratory Values for Patients Based Upon Severity of Illness Classifications

Mean (± SD)	Chronic n = 20	Neg SIRS n = 30	SIRS n = 29	Sepsis n = 13	Severe Sepsis n = 27	Septic Shock n = 16	p Value
Pulse		78 (± 18)	49 (± 56)	58 (± 63)	15 (± 38)	73 (± 60)	0.001
Respiration		16 (± 3)	11 (± 12)	11 (± 12)	3 (± 7)	14 (± 13)	0.001
Min Temperature		36.7 (± 0.8)	36.2 (± 1.5)	36.5 (± 1.3)	36.5 (± 1.1)	36.6 (± 1.4)	0.668
Max Temperature		36.8 (± 0.6)	36.6 (± 1.1)	37.2 (± 1.2)	37.7 (± 1.9)	37.3 (± 1.2)	0.098
MAP		84.4 (12.9)	84.2 (± 21.0)	92.3 (± 16.5)	88.8 (± 19.6)	53.4 (± 6.6)	0.434*
Laboratory Values							
WBC	5.5 (± 1.6)	8.5 (± 3.3)	13.6 (± 11.4)	15.8 (± 4.3)	15.1 (± 9.0)	21.7 (± 19.9)	0.002
Platelets	199 (± 65)	152 (± 62)	117 (± 105)	198 (± 83)	113 (± 76)	92 (± 71)	0.004
Protime		31.0 (± 14.4)	29.1 (± 15.8)	22.8 (± 9.2)	24.5 (± 11.7)	35.7 (± 23.9)	0.130
INR	0.9 (± 0.5)	3.2 (± 1.7)	2.9 (± 1.8)	2.3 (± 1.2)	2.3 (± 1.2)	4.1 (± 2.9)	0.027
Bilirubin	0.4 (± 0.2)	15.6 (± 11.0)	13.0 (± 9.9)	13.5 (± 11.0)	14.5 (± 12.0)	13.9 (± 9.4)	0.919
AST		2231 (± 4190)	1745 (± 3664)	692 (± 1278)	938 (± 1497)	4382 (± 4390)	0.017
ALT		2976 (± 3090)	2270 (± 4698)	1030 (± 718)	1125 (± 1532)	2145 (± 3286)	0.155
Creatinine	0.9 (± 0.1)	1.8 (± 1.6)	2.6 (± 2.0)	1.1 (± 0.28)	2.4 (± 1.8)	3.1 (± 1.1)	0.007
Lactate		8.6 (± 17.4)	8.8 (± 7.7)		21.0 (± 36.4)	27.1 (± 40.8)	0.283
pO2		140 (± 72)	97 (± 31)	145 (± 87)	113 (± 37)	99 (± 40)	0.017
O2 sat		98 (± 2)	93 (± 16)	96 (± 5)	98 (± 2)	92 (± 12)	0.167
pCO2		33.7 (± 4.0)	27.8 (± 7.1)	27.6 (± 1.4)	26.6 (± 6.1)	25.1 (± 5.4)	<0.001
FiO2		38.6 (± 27.3)	37.8 (± 21.9)	32.4 (± 15.6)	41.5 (± 23.4)	58.4 (± 33.3)	0.065
pO2 / FiO2		422 (± 200)	277 (± 149)	456 (± 159)	270 (± 113)	287 (± 372)	0.399

*Septic Shock was not included because the MAP level is defined by the category

the analysis because the MAP values were inclusion/exclusion criteria for the category. Parameters from the laboratory data that were significantly different included WBC (p-value = 0.002), platelet (p-value = 0.004), INR (p-value = 0.027), AST (p-value = 0.017), creatinine (p-value = 0.007), pO2 (p-value = 0.017), and pCO2 (p-value < 0.001).

Statistical Results

Because the PCT values were not normally distributed, the PCT values were transformed using the following equation: $\text{Log}_{10}(\text{PCT} + 1)$. The horizontal line in Figure 5 represents the 2.0 ng/mL cut-off that is indicative of severe sepsis in the literature (Harbarth et al., 2001). As can be seen, the distribution of SPCTC results of the different severity categories vary greatly. Because the assumption of equal variances among

المنارة للاستشارات
www.manaraa.com

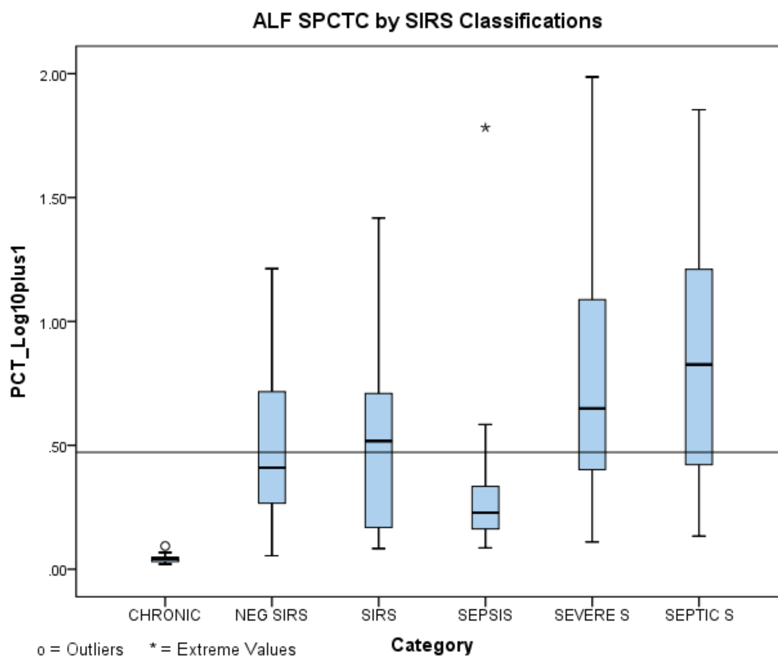

Figure 5: SPCTC by SIRS Classifications

This graph represents the median transformed SPCTC values for the six patient severity groups. The horizontal line represents the transformed 2.0 ng/mL SPCTC cutoff value indicative of severe sepsis.

groups for an ANOVA was violated, the non-parametric Kruskal-Wallis test was used to address the following hypotheses for Specific Aim 1.

H_0: There is no difference in SPCTCs between non-infected ALF/ALI patients and non-infected chronic liver disease patients.

The Kruskal-Wallis test indicated a significant difference between the three non-infection groups (chronic, Neg SIRS, and SIRS) with a $\chi^2 = 43.682$ (df = 2, p-value <0.001). Using the post hoc multiple comparisons tests with the Dunn Method (Elliot & Hynan, 2011), there is a significant difference between the chronic group and the Neg SIRS (p-value <0.001) and the SIRS (p-value <0.001) groups. However, there was no significant difference between the Neg SIRS and SIRS categories (p-value >0.05).

المنارة للاستشارات

www.manaraa.com

The next hypothesis addressed the comparison of non-infected subjects to the SPCTC reference value:

H_0: There is no difference in SPCTSs between non-infected patients with liver disease (chronic and ALF/ALI) and the published SPCTC reference range (<0.1 ng/mL) for non-infected patients in the general population.

For this hypothesis, a one sided t-test was used to determine if there were differences between the SPCTC results for all non-infected patients and the documented reference range (<0.1 ng/mL) for non-infection. When all of the non-infected patients (chronic, Neg SIRS, and SIRS) were tested, the mean transformed SPCTC (tSPCTC) value was 0.393 (SPCTC = 1.472 ng/mL). This mean was significantly different from the null hypothesis value of 0.1 (based upon the package insert reference range value for non-infected patients in the general population of <0.1 ng/mL) with a t value of 8.741 (df = 79, p-value <0.001). When the single outlier in the chronic patients group was removed from the data and the analysis was repeated, the p-value was still <0.001. However, when each category was analyzed individually, the results for patients with chronic liver disease were not significantly different from the published reference range, while results from each of the other two categories (Neg SIRS and SIRS) were significantly different (Table 14).

The final Specific Aim 1 hypothesis addressed the comparison of the five severity categories:

www.manaraa.com

Table 14: Comparison of tSPCTC in Non-Infected Patients to the Published Reference Value (<0.1 ng/mL)

Category	Mean (ng/mL)	SD	df	t statistic	1-sided p-value
All	0.393	0.357	78	8.741	<0.001
Chronic	0.043	0.017	19	0.511	0.307
Neg SIRS	0.510	0.327	29	7.854	<0.001
SIRS	0.512	0.358	28	7.089	<0.001

H_0: There is no difference in SPCTC between ALF/ALI patients with different degrees of severity of infection (i.e., no infection, SIRS, sepsis, severe sepsis, and septic shock).

Kruskal-Wallis with post hoc testing (Dunn Method) was used to evaluate the five ALF patient categories. The Kruskal-Wallis test showed that overall there was a significant difference in SPCTC among the five categories (χ^2 = 15.583, df = 4, p-value = 0.003). However, in Table 15, the mean ranks of each of the five categories indicate that the significant differences were found only between the sepsis category and the severe sepsis (p-value <0.01) and septic shock categories (p-value <0.05).

Table 15: Results for post hoc Pairwise Testing Using the Dunn Method

Category		N	Mean Rank	p-value			
No.	Name			vs 2	vs 3	vs 4	vs 5
1	Neg SIRS	30	53.60	>0.05	>0.05	>0.05	>0.05
2	SIRS	29	51.83		>0.05	>0.05	>0.05
3	Sepsis	13	34.62			<0.01	<0.05
4	Severe Sepsis	27	71.63				>0.05
5	Septic Shock	16	73.44				

Subjects in the sepsis, severe sepsis, and septic shock groups were examined based upon types of cultures: blood cultures vs. all other culture types. A 2-way analysis

المنارة للاستشارات
www.manaraa.com

of variance was performed to examine the difference in SPCTC by type of culture (blood vs. all other culture types), severity categories (sepsis, severe sepsis, and septic shock), and the interaction of these two factors. The interaction between the type of culture and severity categories (F = 0.098, dfn = 1, dfd = 50, p-value = 0.906) and the type of culture category (F = 1.419, dfn = 1, dfd = 50, p-value = 0.239) were non-significant. However, there was a significant difference between the severity categories (F = 3.627, dfn = 2, dfd = 50, p-value = 0.034). While there was a difference between the transformed means for severity, there is no difference between the SPCTC levels for subjects with positive blood cultures and positive non-blood cultures.

Because the sepsis category did not, by definition exist in the ALF population, SPCTC results were examined with the sepsis severity category combined with the severe sepsis severity category. When the SPCTC results were examined without the sepsis severity category, there were no significant differences between the categories (χ^2 = 5.038, df = 3, p-value = 0.169).

The Neg SIRS and SIRS categories that were presumed to have no infection demonstrated mean SPCTCs at or above the 2.0 ng/mL cut-off and while the Sepsis category which had documented infections had a mean SPCTC that was below the 2.0 ng/mL cut-off. For this reason, there was still some uncertainty as to the actual cut-off value denoting the presence of infection was for this population. To address this, the SPCTC data from all categories were re-categorized based upon infection and non-infection and a ROC analysis was performed. Samples were entered into the analysis listwise and the coordinates of the ROC curve were generated based upon sensitivity and

المنارة للاستشارات
www.manaraa.com

1-specificity at each value for SPCTC (Figure 6). The sensitivity indicates the percentage of samples correctly identified as infected when they are actually infected. The specificity is the percentage of samples correctly identified as not infected when they do not have an infection. The tests results for the ROC produced an area under the curve (AUC) equal to 0.697 (SE = 0.044, p-value <0.001). Upon examining the results of the coordinates of the curve, it was determined that the best cut-off value for this set of data, using a combination of criteria, was 1.62: 1) the point on the curve that was at the greatest distance (0.186) from the 0.5 reference line at a 45° angle to the line, 2) accuracy, 3) sensitivity (0.643), 4) specificity (0.620), and 5) likelihood ratio (1.693).

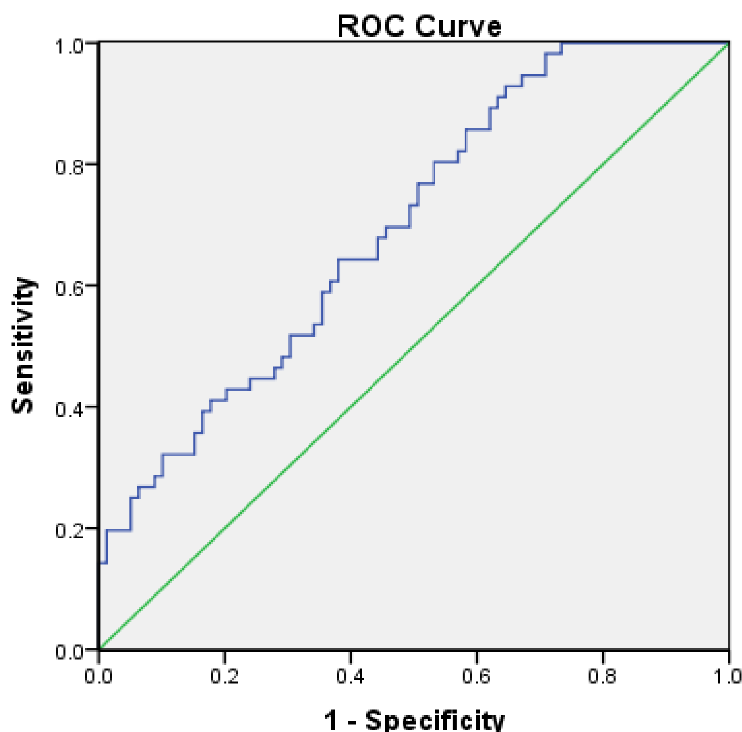

Figure 6: Receiver Operator Curve for the Detection of Infection in ALF and ALI patients Using SPCTC

The ROC analysis resulted in an AUC of 0.697 with a sensitivity of 64.3% and a specificity of 62.0% for the use of PCT in the detection of infection this population of ALF and ALI patients.

المنارة للاستشارات
www.manaraa.com

When the biodata between the ALF subjects with a SPCTC, that was less than the 1.62 ng/mL cut-off and those with a SPCTC that was greater than or equal to the cut-off are compared, with the exception of creatinine, AST, ALT, and bilirubin, all biodata parameters had similar results (Table 16). It is uncertain how the differences in these laboratory results impacted the SPCTC values, but they were noted. However, there are several features of note about the ≥ 1.62 ng/mL group. First, 52.2% of the subjects in the ≥ 1.62 ng/mL group had a diagnosis of acetaminophen toxicity, which was 84.5% of all APAPs in the study. This raised a question as to whether APAP toxicity and the resulting liver damage it caused was a factor in the increased SPCTC. In addition, the majority (65.0%) of subjects in the Neg SIRS and SIRS categories had an outcome of death or received a liver transplant. This will be discussed more fully later, in the discussion of Specific Aim Three.

Fifty percent of all ALF cases in the ALFSG database are a result of APAP toxicity. To ensure an equal distribution of etiology types in this section of the study, equal numbers of samples were requested for each of three etiology types: acetaminophen toxicity, viral hepatitis, and all other etiologies. An interesting trend was noted when SPCTC results were examined based upon etiology. The SPCTC median values for subjects with APAP toxicity were higher than the other etiologies in all of the severity categories (Figure 7). The median SPCTC results for the severity categories with APAP etiology were 3.0 to 6.6 times higher than the results for the respective severity categories for all other etiologies (Table 17), again suggesting that patients with APAP toxicity may have elevated SPCTC values unrelated to infection. However, the patterns

المنارة للاستشارات
www.manaraa.com

Table 16: Demographic and Biodata Results Based Upon the Calculated Infection Cut-off Value of 1.62 ng/mL

Mean (± SD) or Number (%)	SPCTCs ≥ 1.62 ng/mL n = 67	SPCTCs < 1.62 ng/mL n = 48	p-value
ALF Etiology			
APAP	35 (52.2)	6 (12.5)	
Viral hepatitis	11 (16.4)	17 (35.4)	
Other	21 (31.3)	25 (52.1)	
Outcome (TFS)			
APAP	21(31.3)	3 (6.2)	
Viral Hepatitis	3 (4.5)	5 (10.4)	
Other	6 (9.0)	5 (10.4)	
WBC	13.7 (± 9.7)	14.4 (± 12.9)	0.732
Platelet	120 (± 91)	146 (± 76)	0.104
INR	2.8 (± 1.7)	3.1 (± 2.1)	0.409
Glucose	149.0 (± 97.7)	121.2 (± 43.3)	0.215
Creatinine	2.8 (± 1.9)	1.4 (± 0.8)	0.000
AST	2828 (± 4142)	739 (± 1862)	0.001
ALT	2870 (± 3873)	1179 (± 1925)	0.006
Lactate	17.4 (± 30.2)	7.2 (± 7.5)	0.222
Bilirubin	10.4 (± 9.9)	19.2 (± 9.7)	0.000
pO2	111.3 (± 41.9)	120.3 (± 68.5)	0.428
pCO2	27.7 (± 7.1)	28.8 (± 4.8)	0.388
Pulse	51 (± 49)	68 (± 45)	0.108
Respiration	11 (± 10)	14 (± 9)	0.204
Min Temp	36.6 (± 1.2)	36.5 (± 0.9)	0.663
Max Temp	37.2 (± 1.3)	36.9 (± 0.8)	0.303
Coma Grade			
0 / Not reported	4	3	
1	9	7	
2	9	11	
3	12	9	
4	33	18	
Means were calculated based upon the available data			

المنارة للاستشارات
www.manaraa.com

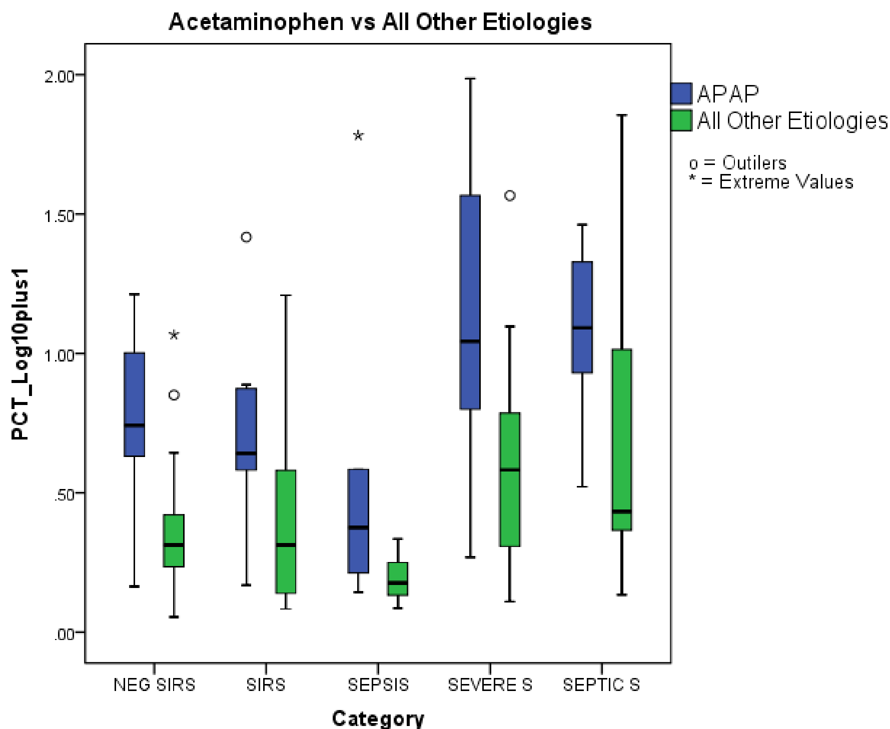

Figure 7: Median SPCTCs by SIRS Categories Sorted by Etiologies

This graph represents the median transformed SPCTC values for the five patient severity groups sorted by etiologies: APAP and All Others etiologies (combined viral and other).

Table 17: Comparison of Median SPCTC Results of Subjects in Specific Aim One Sorted by Category and Etiologies

	Neg SIRS		**SIRS**		**Sepsis**		**Severe Sepsis**		**Septic Shock**	
	N	Median (ng/mL)	N	Median (ng/mL)	N	Median (ng/mL)	N	Median (ng/mL)	N	Median (ng/mL)
All Etiologies	30	1.57	30	2.29	13	0.69	26	3.46	16	5.89
APAP	10	4.53	10	3.38	6	1.48	10	11.34	5	11.37
All Other Etiologies	20	1.05	20	1.07	7	0.50	16	2.41	11	1.71

of the results for the APAP and All Other Etiologies groups remain same as the overall data: SPCTC results in the Neg SIRS and SIRS severity categories were higher than those of the sepsis category, while the Severe Sepsis and Septic Shock categories had the SPCTC highest levels. When Kruskal-Wallis statistical analyses were performed on the

المنارة للاستشارات
www.manaraa.com

two etiology groups (APAP vs. all other etiologies), there were significant differences among the severity categories for the All Other Etiologies group with a χ^2 = 12.942 (df = 4, p-value = 0.012). However, there were no significant differences among the severity categories for the APAP etiology group with a χ^2 = 8.515 (df = 4, p-value = 0.074). The patterns of the mean ranks for each subgroup were similar to the pattern of the mean ranks for all subjects (Figure 8).

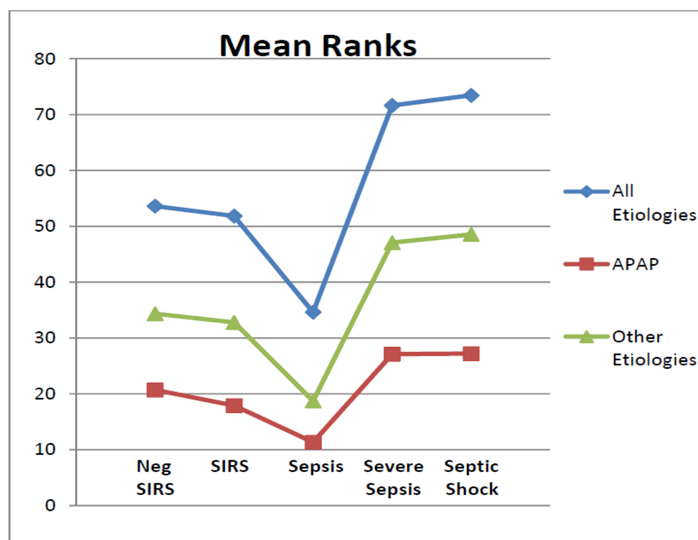

Figure 8: Comparison of Kruskal-Wallis Mean Ranks for All, APAP, and Other Etiologies

The patterns of the mean ranks of the APAP and Other Etiologies across the five categories were similar to the pattern for all subjects. The levels of the three etiology groupings are different due to the differing number of subjects in each grouping.

A ROC analysis was performed on non-APAP subjects only, the AUC was 0.612 (SE = 0.066, p-value = 0.099). Upon examining the results of the coordinates of the curve, it was determined that the best cut-off value for the non-APAP data was 1.62, with a sensitivity of 0.543 and a specificity of 0.692.

www.manaraa.com

Specific Aim Two

The purpose of Specific Aim 2 was to determine the utility of SPCTC as a screening biomarker for infection in ALF patients. To accomplish this, subjects with a confirmed infection and with samples available on the day of infection and the three days prior to the day of infection were tested to determine the SPCTC values. Results were analyzed to determine if SPCTC results increased relative to the day of infection and on which day – DoI, Day -1, Day -2 or Day -3.

Sample Selection

From the 1863 patients that were available from the ALF and ALI databases, 130 subjects had an infection identified on Days 4, 5, 6, or 7 without a confirmed infection on the prior days. A list of these subjects was given to the Program Manager to randomly select 45 subjects who had samples available for each of the four days required for this portion of the study (DoI and 1, 2, and 3 days prior to DoI). A total of 34 subjects met these criteria.

Demographic Information

These subjects had a mean age of 40.2 years (SD = 13.9, median = 41.0, range 18 to 73), were 61.8% female, and 88.2% Caucasian. The proportion of etiologies represented (APAP = 44.1%, viral hepatitis = 8.8%, and all other etiologies = 47.1%) were similar to that described by the USALFSG overall for the ALF and ALI studies (APAP – 50%, viral hepatitis – 12%, and all other causes 38%) (Lee & Seremba, 2008; United States Acute Liver Failure Study Group, 2011). The types of cultures that were positive for bacterial growth were equally distributed between blood (35.3%), tracheal

www.manaraa.com

aspirates (29.4), and urine (32.3%) cultures. There was a single positive catheter culture (2.9%). Within the blood and tracheal aspirate cultures, seven subjects had multiple positive culture sites on the DoI (20.5% of total). Table 18 provides the demographic information for the patients included in the analysis for Specific Aim Two.

Statistical Results

When all SPCTC values for each subject (Days -3, -2, -1, and DoI) were plotted, the concentrations vary greatly across all days (Figure 9). There is no readily apparent overall trend to the values. The median values for all days are in fact very similar, as can be seen from the box plots in Figure 10. A Friedman test for non-parametric related samples was used to test the hypothesis for this part of the study.

H_0: There is no difference in SPCTC in ALF/ALI patients between the first day a positive culture was collected and the three days prior to a positive culture.

The results of the analysis gave a $\chi^2 = 6.741$ (n = 34, df = 3, p-value = 0.081) indicating no significant difference in the PCT concentrations for the four days.

Because there appeared to be some patterns within the samples, the samples were sorted based upon the Day -3 (D-3) and DoI serum PCT concentrations.

- Subgroup A – D-3 samples were greater than the 3.0 ng/mL
- Subgroup B – D-3 samples were less than 3.0 ng/mL and had an increase by DoI
- Subgroup C – D-3 samples were at or below the 1.62 ng/mL cutoff and SPCTC values remained below the 1.62 ng/mL cut-off or decreased by DoI

المنارة للاستشارات
www.manaraa.com

Table 18: Demographic Information for 34 Patients Analyzed for SPCTC to Detect Bacterial Infection

Mean (± SD) or Number (%)	n = 34
Gender	
Female	21 (61.8)
Male	13 (38.2)
Race / Ethnicity	
Hispanic	4 (11.8)
Caucasian	30 (88.2)
African-American	1 (2.9)
Asian	1 (2.9)
Native American	2 (5.9)
Age (years)	40.2 (± 13.9)
Sample age (years)	8.3 (± 2.7)
Days In Hospital Prior DoI	8.0 (± 5.0)
Etiologies	
Acetaminophen	15 (44.1)
Viral Hepatitis	3 (8.8)
All others	16 (47.1)
Outcomes (TFS)	
Acetaminophen	11 (32.4)
Viral Hepatitis	0 (0.0)
All others	7 (20.6)
Culture types	
Blood culture -1	6 (17.6)
Tracheal aspirates - 2	9 (26.5)
Urine - 3	11 (32.4)
Catheter - 5	1 (2.9)
*Multiple w Blood – 7	6 (17.6)
**Multiple wo Blood – 8	1 (2.9)
Antibiotic	
Prophylaxis	11 (32.4)
Antibiotic Therapy	20 (58.8)
Antibiotics Used	25 (73.5)
Coma Grade (DoI)	
Not Reported/0	8 (23.5)
1	6 (17.6)
2	6 (17.6)
3	4 (11.8)
4	10 (29.4)

*Blood culture in combination with other culture types
** Multiple culture types in combinations that do not include blood cultures

www.manaraa.com

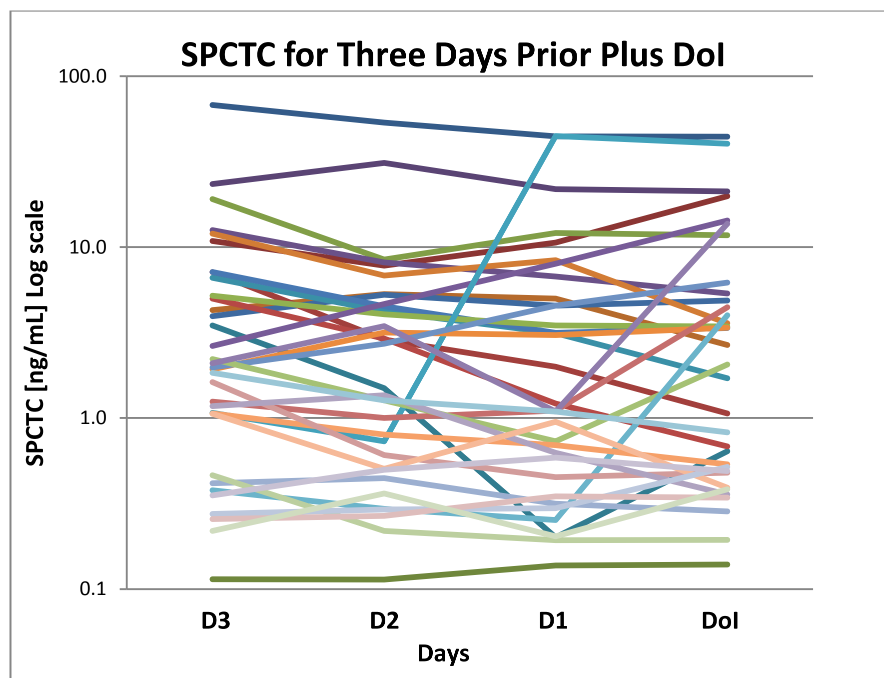

Figure 9: SPCTC Results for All Subjects for Three Days Prior to and the Day of Infection

Each series of results represents one subject's SPCTC results for Day of Infection (DoI), one Day prior to DoI (D-1), two Days Prior to DoI (D-2), and three Days prior to DoI (D-3).

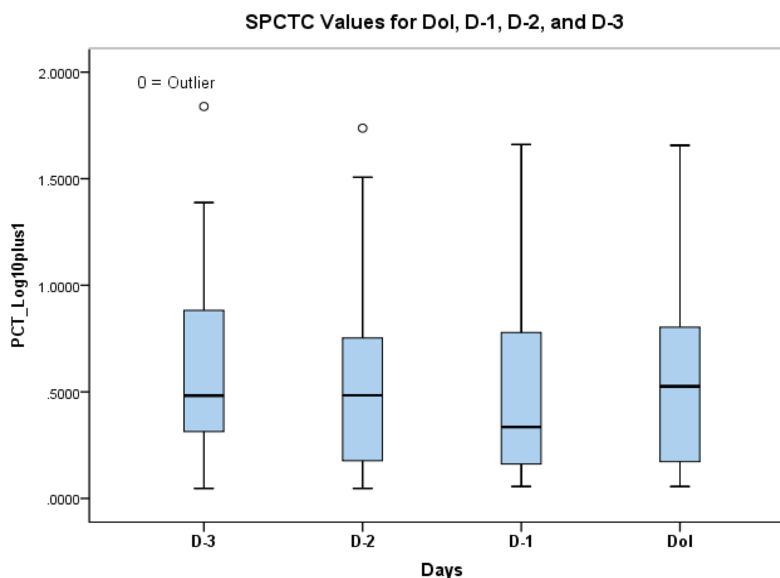

Figure 10: SPCTC Values for DoI and Three Days Prior to DoI

The lines within the box represent the medians for the transformed SPCTC values for the Day of Infection (DoI) and three days prior to DoI.

المنارة للاستشارات
www.manaraa.com

The 3.0 ng/mL break between Groups A and B was chosen because it was centralized in a nearly 1.0 ng/mL gap between the lowest Group A sample (3.48 ng/mL) and the highest Group B sample (2.65 ng/mL) when the D-3 SPCTC values were placed in rank order. Rank ordered D-3 SPCTC values for Groups B and C were intermixed. To help sort these groups SPCTC values for the DOI were examined and values that increased were assigned to Group B and those values that remained below the 1.62 ng/mL cut-off were assigned to Group C.

In Subgroup A (Figure 11), all of the SPCTCs on D-3 were above 5.0 ng/mL with the exception of one sample that was 3.95 ng/mL. All subjects showed a general decrease in SPCTC by DoI.

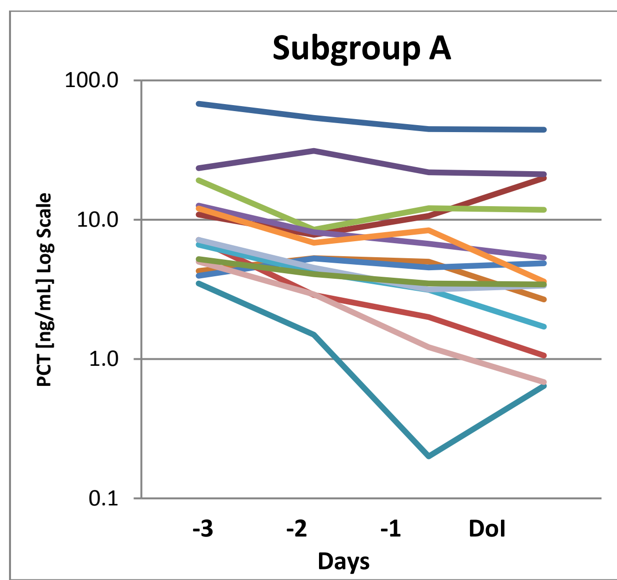

Figure 11: SPCTC Results for Subjects in Subgroup A

Subjects SPCTC results in Subgroup A were all greater than 3.0 ng/mL on D3 prior to Day of Infection. The majority showed a decrease in the SPCTC by DoI.

المنارة للاستشارات

www.manaraa.com

Subgroup B (Figure 12) had SPCTCs on D-3 that were <3.0 ng/mL and increased by DoI. While the SPCTC values for some subjects initially decreased, all subjects had SPCTC values that increase above the 3 ng/mL D-3 level on or before the DoI.

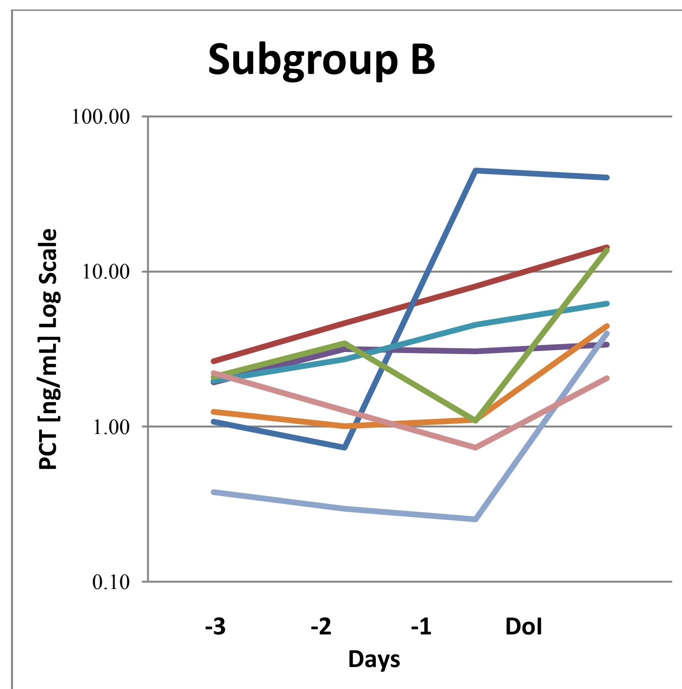

Figure 12: SPCTC Results for Subjects in Subgroup B

Subjects SPCTC results in Subgroup B were all less than 3.0 ng/mL on D3 prior to Day of Infection. All SPCTC results increased by DoI to values greater than 3 ng/mL.

Subgroup C (Figure 13) had SPCTCs on D3 that were less than or equal to the 1.62 ng/mL cutoff with the exception of one subject that had a D-3 SPCTC of 1.84 ng/mL. All subjects' SPCTC results were less than 1.0 ng/mL by DoI.

There were no apparent differences in the demographic characteristics among three subgroups and all subjects on the DoI with the exception of Subgroup B, which had an increased WBC. Other variables such as lactate, pCO2, pulse, respiration, and

المنارة للاستشارات
www.manaraa.com

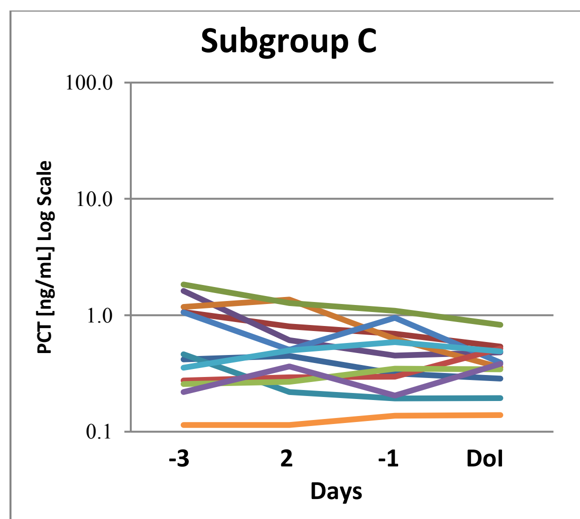

Figure 13: SPCTC Results for Subjects in Subgroup C

Subjects SPCTC results in Subgroup C were all less than the 1.62 ng/mL cut-off on D3 prior to Day of Infection. The SPCTC results remained below the 1.62 ng/mL cut-off or decreased by DoI.

temperature were not calculated because in most cases there were a large number of missing values (Table 19).

A Friedman Test was used on each of the subsets of subjects to determine if there were any significant differences in the SPCTC results among each of the four days tested (Table 20). The subjects from Subgroup A showed a significant change between days with a $\chi^2 = 18.257$ (df = 3, p-value <0.001). These patients had a decrease in the median SPCTC values from 7.16 ng/dL on D-3 to 3.40 ng/dL on DoI for an overall decrease in the mean SPCTC values of 52.5%. Using a Wilcoxon Signed Ranks test, the change between D-3 and DoI was significant (p-value 0.013, Z = -2.480). The differences between Day -2 (D-2) and Day -1 (D-1) and the DoI were not significant (D-2 p-values = 0.74, D-1 p-value = 0.096). While two (14.3%) of the subjects in this group did not receive antibiotics (prophylaxis or therapy), four (28.6 %) received prophylactic

www.manaraa.com

Table 19: Demographic Data for All Subjects and Each of the Three Subgroups on the Day of Infection

Mean (± SD) or Number (%)	All n = 34	Subgroup A n = 14	Subgroup B n = 8	Subgroup C n = 12
Days in Hospital Prior to D-3	Mean = 3.18 Median = 2	Mean = 4.14 Median = 2	Mean = 2.13 Median = 1	Mean = 2.75 Median = 2
Etiologies				
Acetaminophen	15 (44.1)	10 (71.4%)	2 (25.0)	3 (25.0)
Viral Hepatitis	3 (8.8)		1 (12.5)	2 (16.7)
Other	16 (47.1)	4 (28.6%)	5 (62.5)	7 (58.3)
Outcome (TFS)				
APAP	11 (32.4)	9 (64.3)	1 (12.5)	1 (8.3)
Viral Hepatitis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other	7 (20.6)	3 (21.4)	2 (25.0)	2 (16.7)
WBC	13.8 (± 11.4)	12.7 (± 6.6)	20.2 (± 20.0)	10.6 (± 5.5)
Platelet	85 (± 58)	98 (± 66)	66 (± 37)	114 (± 55)
INR	2.2 (± 1.4)	1.7 (± 0.6)	1.8 (± 0.5)	3.0 (± 2.0)
Bilirubin	14.9 (± 9.8)	13.1 (± 8.1)	15.4 (± 11.7)	16.9 (± 10.8)
AST	352 (± 378)	245 (± 207)	380 (± 467)	486 (± 474)
ALT	687 (± 630)	858 (± 709)	430 (± 475)	668 (± 613)
Creatinine	2.3 (± 2.0)	2.9 (± 2.0)	3.2 (± 2.6)	0.9 (± 0.5)
Coma Grade				
0 / Not reported	8 (23.5)	4 (28.6)	2 (25.0)	2 (16.7)
1	6 (17.6)	2 (14.3)	2 (25.0)	2 (16.7)
2	6 (17.6)	3 (21.4)	1 (12.5)	2 (16.7)
3	4 (11.8)	1 (7.1)	1 (12.5)	2 (16.7)
4	10 (29.4)	4 (28.6)	2 (25.0)	4 (33.3)
Antibiotics				
Used	25 (73.5)	12 (85.7)	4 (50.0)	9 (75.0)
Prophylaxis	11 (32.4)	4 (28.6)	3 (37.2)	4 (33.3)
Treatment	20 (58.8)	10 (71.4)	4 (50.0)	6 (50.0)
Culture types				
Blood culture -1	6 (17.6)	1 (7.1)	3 (37.5)	2 (16.7)
Tracheal aspirates - 2	9 (26.5)	4 (28.6)	3 (37.5)	2 (16.7)
Urine - 3	11 (32.4)	5 (35.7)	1 (12.5)	5 (41.7)
Catheter - 5	1 (2.9)	1 (7.1)		
*Multiple w Blood – 7	6 (17.6)	3 (21.4)	1 (12.5)	2 (16.7)
**Multiple wo Blood – 8	1 (2.9)			1 (8.3)

*Blood culture in combination with other culture types
** Multiple culture types in combinations that do not include blood cultures

www.manaraa.com

Table 20: Friedman Test for SPCTC Results for All Samples and Subgroups A, B, and C

Median Mean Rank	N	D-3	D-2	D-1	DoI	Chi-Square	df	p-value
All samples	34	2.04 2.91	2.11 2.62	1.16 2.18	2.37 2.29	6.741	3	0.081
Subset A	14	7.16 3.50	5.28 2.86	4.78 2.07	3.40 1.57	18.257	3	<0.001
Subset B	8	1.96 2.13	1.99 2.00	2.09 2.13	5.34 3.75	10.050	3	0.018
Subset C	12	0.44 2.75	0.47 2.75	0.40 2.33	0.39 2.17	1.900	3	0.593

antibiotics and twelve (85.7 %) received antibiotics (therapy or prophylaxis). The two subjects who did not receive antibiotics had the highest and the lowest values for all days in this group and had documented urinary tract infections on DoI. Also of interest in this group is the large number of APAP cases. It was noted earlier, the SPCTC results of APAP cases appeared to have increased levels compared to the SPCTC levels for the other etiologies. This may be influencing the results in this subgroup irrespective of any evidence of infection.

Patients from Subgroup B also showed a significant change in the SPCTC values between days. The median SPCTC values increased from 1.96 ng/mL on D-3, 1.99 ng/mL on D-2, 2.09 ng/mL on D-1 to 5.34 ng/mL on DoI. The Freidman test gives a $\chi^2 = 10.050$ (df = 3, p-value = 0.018). The Wilcoxon Signed Ranks test showed significant differences between DoI and both D-3 and D-2 (Z = -2.380, p-value = 0.017 and Z = -2.521, p-value = 0.012, respectively). No other pairs were significant.

Patients from Subgroup C had median SPCTC values for all days that were below the 1.62 ng/mL cutoff. In fact there were only two values from the whole group (n = 12)

المنارة للاستشارات
www.manaraa.com

that were at or above the cutoff on D-3 but decreased below the cutoff by D-2 and remained there. Both patients had confirmed bacterial positive cultures on tracheal aspirates (one also had a positive blood culture) and both received antibiotics. There were 12 subjects in C group of which 75% received antibiotics (prophylaxis or therapy). Urine cultures accounted for 41.7% of the positive bacterial cultures followed by 33.4% blood cultures and 25.0% tracheal aspirate cultures.

Specific Aim Three

The purpose of Specific Aim 3 was to determine if changes in the SPCTC in ALF and ALI patients, with a confirmed bacterial infection on Day 1, would predict which of two outcomes these patients would achieve – 1) transplant free survival or 2) death or transplant.

Sample Selection

Of the 1863 available ALF and ALI subjects, 259 subjects had a documented positive culture on Day 1, of which 207 had a positive culture on day 1 only. The 52 subjects with cultures on multiple days were eliminated to avoid a potentially confounding variable. The sample list was sorted by outcome (TFS or DoT) and the list was given to the Project Manager. Samples from 30 subjects from both outcome groups, TFS and DoT, with serum samples for each of the seven days of the ALF or ALI studies were requested. It was determined that there were too few subjects in either group with all seven samples available. There are several reasons that samples might be missing that included:

المنارة للاستشارات

www.manaraa.com

- Subjects no longer enrolled in the study due to death, transplant, discharge, or withdrawal of consent
- ALF and ALI studies are a non-treatment study that collects clinical data and samples if available
- Sample aliquot inventory from individual days may have been depleted and therefore unavailable

The requirements were adjusted to include subjects based upon the following minimum requirements for each subject:

- Days 1 – 4 samples were available
- Single days could be missing if more than 5 days were available
- Missing days could not be Day 1 or any consecutive days (except at the end of the series)

The final available sample sets were 26 subjects for the TFS group and 21 subjects for the DoT group. Table 21 shows the number of available results for each group by day. As can be seen, the number of available samples decreases on Days 5, 6, and 7 for both groups but the decrease is greater in the DoT group.

Table 21: Number of Samples Tested by Days and Outcomes

	N	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7
TFS	26	26 (100%)	26 (100%)	25 (96.2%)	23 (88.5%)	22 (84.6%)	18 (69.2%)	16 (61.5%)
DoT	21	21 (100%)	20 (95.2%)	20 (95.2%)	21 (100%)	13 (61.9%)	9 (42.9%)	6 (28.6%)

المنارة للاستشارات
www.manaraa.com

Demographic Information

Demographic information for both groups (TFS and DoT) is described in Table 22. Both groups were similar in all characteristics described except the etiology of ALF. The TFS group had a higher percentage of APAP subjects (73.1%) compared to DoT group (19.0%), while the DoT group had a higher percentage of Other etiologies (76.2%) compared to APAP (23.1%).

A comparison of the laboratory values for TFS and DoT on DoI (see Table 23) indicated that except for bilirubin and lactate (which are higher in the DoT group) the two groups were similar.

Statistical Results

The SPCTC values ranged from 0.13 ng/mL to 103.75 ng/mL. Because of the non-normally distributed SPCTC results and the large number of missing samples, a mixed models analysis of covariance was used to test the longitudinal hypotheses. This model has two assumptions regarding the sample data: 1) the sample results are normally distributed and have equal variances and 2) the missing data are randomly missing. Because the SPCTC results were widely distributed, the SPCTC values were transformed using the following formula: Log_{10} (SPCTC +1) = transformed SPCTC (tSPCTC) to address the first assumption. One was added to the SPCTC so that SPCTC results transformed using Log_{10} would normalize the variances without introducing negative numbers into the data set. Figure 14 shows the box plot representation of both categories of tSPCTCs for all seven days. Days five, six, and seven had the largest number of

www.manaraa.com

Table 22: Demographic Information Based on Outcomes for Patients with a Documented Infection on Day of Admission to the ALF or ALI Studies

Mean (± SD) or No. (%)	TFS (n = 26)	DoT (n = 21)
Gender		
Female	20 (76.9)	16 (76.2)
Male	6 (23.1)	5 (23.8)
Race / Ethnicity		
Hispanic	0	2 (9.5)
Caucasian	23 (88.5)	16 (76.2)
African-American	2 (7.7)	3 (14.3)
Native American	0	1 (4.8)
Native Hawaiian / Pacific Islander	1 (3.8)	0
Other	0	1 (4.8)
Age (years)	40.6 (± 14.5)	44.8 (± 13.9)
Sample age (years)	6.5 (± 2.9)	7.4 (± 2.6)
Days In Hospital Prior DoI	2.5 (± 2.0)	5.6 (± 10.0)
Etiologies		
Acetaminophen	19 (73.1)	4 (19.0)
Viral Hepatitis	1 (3.8)	1 (4.8)
All others	6 (23.1)	16 (76.2)
Culture types		
Blood culture - 1	4 (15.4)	4 (19.0)
Tracheal aspirates - 2	5 (19.2)	4 (19.0)
Urine – 3	9 (34.6)	7 (33.3)
Ascites - 6	1 (3.8)	1 (4.8)
*Multiple w Blood – 7	3 (11.5)	4 (19.0)
**Multiple wo Blood – 8	4 (15.4)	1 (4.8)
Antibiotic		
Antibiotics Used	18 (69.2)	18 (85.7)
Prophylaxis	8 (30.8)	12 (57.1)
Antibiotic Therapy	15 (57.7)	14 (66.7)
Coma Grade ***		
1	6 (23.1)	3 (14.3)
2	7 (26.9)	6 (28.6)
3	6 (23.1)	5 (23.8)
4	7 (26.9)	7 (33.3)

*Blood culture in combination with other culture types
** Multiple culture types in combinations that do not include blood cultures
***Based on Day 1 Coma Grade

المنارة للاستشارات
www.manaraa.com

Table 23: Laboratory Values for Patients with a Documented Infection on the Day of Admission to the ALF or ALI Studies

Mean (± SD)	TFS (n = 26)	DoT (n = 21)	p-value
WBC	8.5 (± 3.8)	14.4 (± 15.1)	0.060
Platelet	148 (± 95)	183 (± 132)	0.300
INR	3.5 (± 3.4)	2.9 (± 1.8)	0.410
Bilirubin	7.4 (± 6.6)	18.0 (± 11.8)	<0.001
AST	3718 (± 4833)	2876 (± 4657)	0.549
ALT	2876 (± 2821)	2392 (± 3763)	0.616
Glucose	132 (± 50)	112 (± 46)	0.183
Creatinine	1.6 (± 1.3)	2.4 (± 1.4)	0.058
Lactate	2.9 (± 2.0)	6.3 (± 4.5)	0.017
pCO2	31 (± 7)	29 (± 10)	0.367
Pulse	101 (± 30)	101 (± 24)	0.964
Respiration	21 (± 7)	20 (± 7)	0.623
Min Temperature	35.5 (± 7.3)	37.0 (± 0.9)	0.373
Max Temperature	35.8 (± 7.4)	37.1 (± 1.1)	0.420

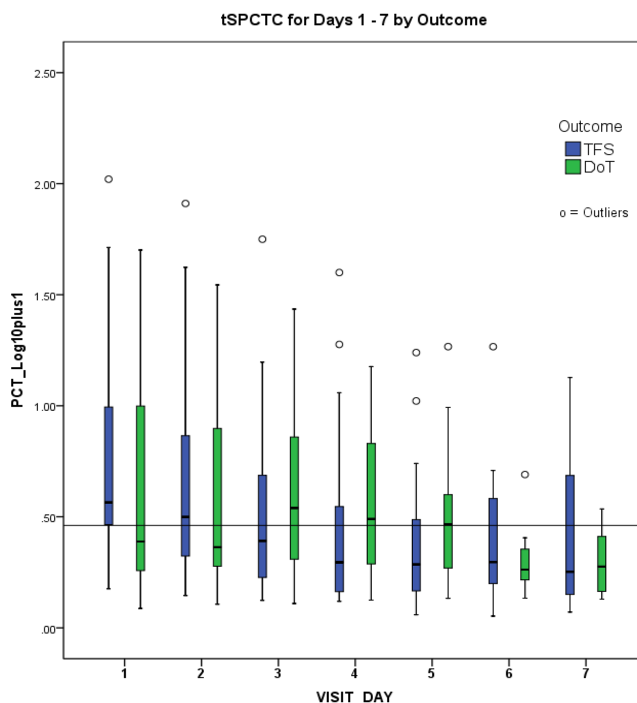

Figure 14: Median tSPCTC per Day Sorted by Outcome

This graph represents the median transformed SPCTC results for Days 1 - 7 for the TFS (blue) and ToD (green) outcome groups.

المنارة للاستشارات
www.manaraa.com

missing samples (see Table 21). Six of 47 subjects had samples missing prior to their last day in the study. All other missing samples were after Day 4. The mixed effects model controls for Type 1 error better than other models when the missing samples rates are different for each group, and the missing samples can be attributed to the outcome (Mallinckrodt, Kaiser, Watkin, Molenberghs, & Carroll, 2004). In this case, the DoT group was missing more samples than the TFS group on days five, six, and seven in large part due to the subjects' death or discontinuation from the study because they received a liver transplant. The mixed model was used to test the hypotheses:

H_0: There is no difference in SPCTC between ALF/ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.

H_0: There is no difference in changes in the serial SPCTCs between ALF/ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.

There appeared to be little difference between the SPCTC results for the TFS subjects and the DoT subjects when the transformed data was examined (Figure 14). However, the TFS group had a larger and faster decrease in the median SPCTC results than the DoT group. The TFS group had a decrease of 66.6% during Days 1 – 5 with an overall decrease by Day 7 of 70.3%. The DoT group that had a 43.7% increase in the median SPCTC values by Day 4 after which the values began falling. The overall decrease in the median SPCTC values for the DoT group (Days 1 through 7) was 49.3%

المنارة للاستشارات
www.manaraa.com

(Table 24). However, it should be noted that by Day 7 there were only 6 of 21 subjects available for analysis in the DoT group compared to 16 of 26 in the TFS group.

Table 24: Change in Medians of SPCTC From Day 1 in TFS and DoT Subjects

	Medians						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
TFS	2.90	2.18	1.49	1.07	0.97	1.12	0.86
Change per day (%)		24.8	31.7	28.2	9.3	-15.5	23.2
DoT	1.42	1.30	2.03	2.04	1.9	0.82	0.72
Change per day (%)		8.5	-56.2	-0.5	6.9	56.8	12.2

To examine differences in the two outcome groups in the longitudinal measurement of SPCTC we considered the following factors: outcome (TFS vs. DoT), visit day, diagnosis (APAP vs. Other Etiologies), severity of illness (sepsis, severe sepsis, and septic shock), use of antibiotics (prophylaxis and/or therapy), antibiotic prophylaxis, antibiotic therapy, coma grade (1-2, mild vs. 3-4, severe), and interactions of these factors. Additional analyses examined those with positive blood cultures ($n = 15$) however, the number of cases was too small to adequately model the data. The final mixed models analysis of covariance with 1-repeated measure (visit days 2, 3, 4, 5, 6, and 7), 2-between groups (outcome and antibiotic use), interaction of outcome * visit day, and baseline SPCTC as a covariate were fit to the data. Baseline covariate of Day 1 SPCTC values was significant ($p<0.0001$). The interaction of outcome * visit day was significant (p-value = 0.0403). After accounting for the baseline covariate, the least square mean estimates for the interaction (Figure 15) show that while the values on Day 2 are similar, the tSPCTC values fell faster in the TFS group than the tSPCTC values in the DoT group. Overall, the TFS SPCTC values were lower than the DoT SPCTC values (p-

المنارة للاستشارات
www.manaraa.com

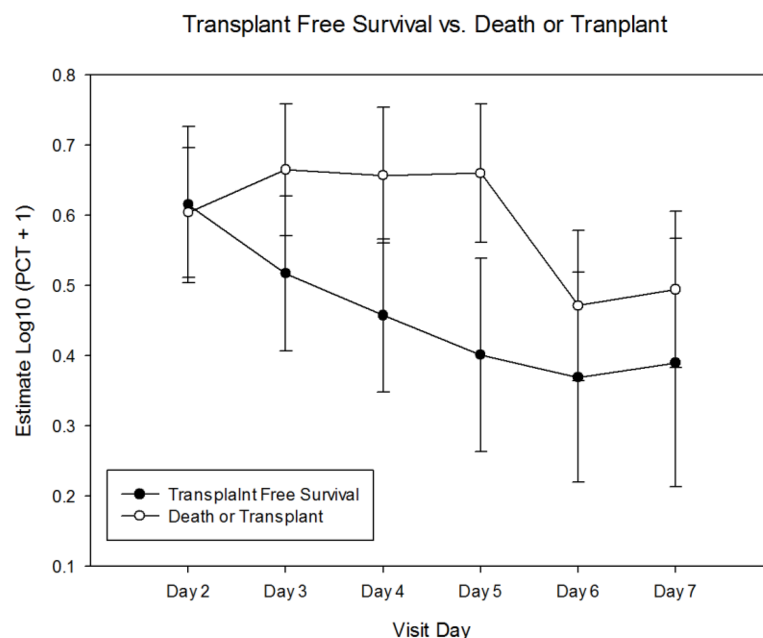

Figure 15: Estimates of Visit Day * Outcome by Day

This graph represents the graphical representation of the estimated least square means of Days 2 – 7 for each of the outcome groups. Day 1 SPCT results are used as a covariate in the statistical calculation of the means. The error bars represent the standard error for each day/outcome.

value = 0.0134) and the SPCTC values decline from Day 2 to Day 7 (p-value = 0.0011). When comparing antibiotic use between the two groups, the TFS group had a SPCTC mean of 0.460 ng/mL (SE $\pm$ 0.027) and DoT group had a SPCTC mean of 0.591 ng/mL (SE $\pm$ 0.049). The two groups were statistically different with a p-value = 0.023.

Summary

This chapter presented the descriptive and statistical analyses for each of the study objectives and hypotheses. Relationships between the serum procalcitonin concentrations and the biodata variables of the ALF and ALI subjects were explored. Procalcitonin did not appear to be a good indicator of infection as it did not adequately detect documented infections in the ALF subjects and was elevated when there was no documented evidence

المنارة للاستشارات
www.manaraa.com

of infection in this study. The data showed that in the presence of infection, SPCTC values increased as the severity of illness (determined by biodata and laboratory results). As noted, there were numerous cases of increased SPCTC values with no documented infection present. The SPCTC results appeared to be affected by physiologic factors associated with various ALF etiologies, particularly APAP toxicity. Changes in SPCTC values over time did appear to be a predictor of outcome with faster and larger decreases in SPCTC values in subjects who survived compared to slower and smaller changes noted for subjects who died or received a liver transplant. Interpretations of the three specific aims will be presented in the next chapter. The interpretations will be related to the results of the literature review.

المنارة للاستشارات

www.manaraa.com

CHAPTER FIVE: DISCUSSION

This chapter presents a review of the results presented in Chapter Four. The results are presented in the context of the listed hypotheses, clinical implications and in comparison to the literature review. Limitations of the study are discussed as well as recommendations for future studies.

Overview of the Problem

Infection and sepsis is a significant problem in the general population with mortality ranging from 10% for patients with SIRS to 50% for patients with septic shock (Brun-Buisson, 2000; Rangel-Frausto et al., 1995). ALF patients are particularly susceptible to infection for several reasons with a reported occurrence of 40% – 90% (Rolando et al., 2000; Vaquero et al., 2003). ALF and ALI patients have impaired immune function, are subject to invasive procedures, and have an increased chance of lung infections when chest physiotherapy and bronchial suction are contraindicated due to cerebral edema. Because of their extremely critical condition, the majority of ALF patients spend at least a portion of their hospital stay in the ICU. Studies have shown that admission to the ICU and use of devices (catheters, ventilators, invasive monitors, etc.) increase the risk of acquiring an infection (Clapperton, Rolando, Sandoval, Davies, & Williams, 1997; Rolando et al., 1990; Rolando et al., 2000; Stravitz, 2008; Suljagic et al., 2005; Wade, Rolando, Philpott-Howard, & Wendon, 2003).

www.manaraa.com

As was noted previously, identification of infection can be difficult and is time consuming. Therefore, finding a method that can quickly and reliably identify a bacterial infection is of great importance. It would be an added benefit if that method could also be used to guide antibiotic therapy. PCT has been shown in the general population to be a reliable marker of infection and is useful as a guide for antibiotic therapy. The purpose of this study was to determine if PCT could be utilized in the acute liver failure and acute liver injury populations to detect infection and by utilizing SPCTC levels, improve outcomes in this difficult situation.

Discussion of the Study

Of the 1683 patients listed in the USALFSG ALF and ALI databases, 632 subjects had an infection identified by standard bacterial culture techniques. This study was undertaken to determine if SPCTC could be used to identify bacterial infections sooner than standard bacterial culture in the ALF and ALI populations. Samples from patients in the ALF and ALI databases were obtained and SPCTC concentrations were measured to answer this question. While a study of SPCTC guided antibiotic therapy could not be accomplished using these retrospective samples, by looking at SPCTC results from serial samples of ALF patients based upon their outcome, this study attempted to determined if SPCTC values for subjects who achieved TFS differed from those who died or received a liver transplant. The study compared the differences in the SPCTC change over time in these two groups of patients.

As can be seen in the summary of the null hypotheses in Table 25, the results of this study were mixed.

المنارة للاستشارات
www.manaraa.com

Table 25: Summary of Study Hypotheses

NULL HYPOTHESES	Expected Results	Actual Results
Specific Aim One		
H_0: There is no difference in SPCTCs between non-infected ALF/ALI patients, and non-infected chronic liver disease patients.	Supported	Rejected
H_0: There is no difference in SPCTSs between non-infected patients with liver disease (chronic and ALF/ALI) and the published PCT reference range (<0.1 ng/mL) for non-infected patients in the general population.	Supported	Rejected
H_0: There is no difference in SPCTC between ALF/ALI patients with different degrees of severity of infection (i.e., Neg SIRS, SIRS, sepsis, severe sepsis, and septic shock).	Rejected	Rejected
Specific Aim Two		
H_0: There is no difference in SPCTC in ALF/ALI patients between the first day a positive culture was collected and the three days prior to the positive culture.	Rejected	Supported
Specific Aim Three		
H_0: There is no difference in SPCTC between ALF and ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.	Rejected	Rejected
H_0: There is no difference in changes in the serial SPCTCs between ALF/ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.	Rejected	Rejected

Specific Aim One

In reviewing the data from this portion of the study, the SPCTC results varied between the different illness severity categories. The chronic liver disease patients' SPCTC values were at or near the expected PCT level of <0.1 ng/mL for "normal" subjects with no infection, partially confirming the hypothesis:

> H_0: There is no difference in SPCTSs between non-infected patients with liver disease (chronic and ALF/ALI) and the published PCT reference range (<0.1 ng/mL) for non-infected patients in the general population.

www.manaraa.com

While SPCTC results from cirrhotic patients in the literature (Bota,et al., 2005; Connert, et al., 2003; Spahr, et al., 2001; Viallon et al., 2000) are mixed, the data from this study indicate that PCT is not elevated in the presence of mild or inactive liver disease. However, the results of ALF subjects, where the liver disease is much more severe, were not as clear.

When looking at ALF subjects across the full SIRS spectrum (Neg SIRS, SIRS, sepsis, sever sepsis, and septic shock) in relation to the hypotheses for this portion of the study, there were significant differences between the severity and etiology categories.

H_0: There is no difference in SPCTCs between non-infected ALF/ALI patients, non-infected chronic liver disease patients.

H_0: There is no difference in SPCTC between ALF/ALI patients with different degrees of severity of infection (i.e., no infection, SIRS, sepsis, severe sepsis, and septic shock).

As noted earlier in chapter four, when looking at the ALF subjects in the severity categories with no documented infection (Neg SIRS and SIRS) in this set of samples, there was a significant difference between both categories and the <0.1 ng/mL reference value and the chronic patients' levels, but no significant difference between the two categories themselves. Thus, the milder severity of illness and presumably infection free categories displayed at least in many instances, **values that approximated those observed in the presence of active bacterial infection**. There were also significant differences between the sepsis category and the severe sepsis and septic shock categories, but not between Neg SIRS, SIRS, severe sepsis, and septic shock.

www.manaraa.com

Within the Neg SIRS and SIRS categories, there were 31 subjects who had SPCTCs of 1.62 ng/mL or greater. This value was determined from the ROC analysis. Of these subjects, 13 had indications of infection later during their hospital/study course that could have affected the SPCTC results. These indications ranged from cultures positive for yeast or fungus, bacterial infections one or more days after the day the tested sample was obtained, and other evidence of infection (i.e., pancreatitis, left lower lobe infiltrate on x-ray, tooth abscess, and VAP). Five subjects had positive bacterial cultures at some point in the seven day course of the ALF or ALI studies after the day the PCT was measured. It was noted earlier that there are many similarities between signs and symptoms associated with ALF and those associated with severe sepsis. These similarities may have resulted in a delay in identifying a bacterial infection. The possible confounders (except yeast/fungal infections) were not captured as a variable in the algorithm used to select samples. Therefore, they were not found until a closer examination of the subject case report forms (CRFs) was made to determine possible causes of the increased SPCTCs. Yeast and fungal infections are captured in the infection data but were eliminated from the dataset as bacterial infection was use as the indication for a positive culture. Five subjects had yeast/fungal infections during the course of the ALF study. There is evidence that yeast and fungal infections can cause an increase in SPCTCs, but these values on average are lower than those seen with bacterial infection (Dornbusch et al., 2005; Martini et al., 2010; Nakamura, Wada, Takeda, & Nobori, 2009).

المنارة للاستشارات
www.manaraa.com

The most interesting feature in this grouping of data was that the sepsis category had a median value of 0.69 ng/mL that was significantly lower than the 1.62 ng/mL calculated cutoff value. Surprisingly, the sepsis group levels were lower than the median values of the Neg SIRS and SIRS categories (1.59 ng/mL and 2.29 ng/mL, respectively). A closer examination of the subject CRFs for this category did not reveal any clear idea as to why the results from this category with proven infections were so much lower than the Neg SIRS and SIRS categories. There were two subjects with tracheal aspirate cultures that were in the category with minimal evidence of infection (yeast and WBCs), but removing them from the category does not change the median value.

All but two of the subjects in the sepsis category (84.6%) received antibiotics, which may have been a factor in the lower SPCTC values for this category. However, all other categories had a high percentage of antibiotics use (Neg SIRS = 63.3%, SIRS = 79.3%, Severe Sepsis = 81.5%, and Septic Shock = 81.2%) as well. There was no difference in antibiotic use across etiologies (APAP, viral, and other) based upon the results of the Fisher's Exact test (p-value = 0.647). It must be noted that the timing of the antibiotic use in relation to the time/day that the serum samples were collected was not known. The study CRFs did not indicate when antibiotic prophylaxis or treatment was initiated or discontinued.

When the results were examined based upon etiology, subjects with APAP toxicity demonstrated higher SPCTC levels than those with any other etiology within each group. Clinically, SPCTC values in subjects with APAP toxicity may not be indicative of infection. There is evidence that APAP toxicity activates macrophages,

المنارة للاستشارات
www.manaraa.com

resulting in the release of cytokines and proinflammatory regulators, including TNFα that contribute to APAP induced liver injury (Dragomir, J. D. Laskin, & D. L. Laskin, 2011; Jaeschke, McGill, & Ramachandran, 2012) . While the exact origin and pathogenic pathway of PCT is still unknown, it has been suggested that PCT is produced by neuroendocrine cells in the liver while other data suggest that it is released by macrophages (Matzaraki, 2007). However, there is some evidence that the increase in PCT may be initiated by TNFα and IL-1b (Whang, 1999; Domensch, 2001). The elevated PCT may be a result of the APAP toxicity itself, but the mechanism of the sterile inflammation remains unclear. The SPCTC levels increased as severity of disease increased suggesting that SPCTC may still provide useful information regarding outcomes for APAP subjects, regardless of the presence or absence of infection. If procalcitonin is to be useful in this population, the cut-off indicating infection may need to be set differently from the cut-offs of the general population and other ALF etiologies. This will have to be examined in future studies.

The median values of the last two categories, severe sepsis (3.46 ng/mL) and septic shock (5.89 ng/mL), were both well above the 1.62 ng/mL calculated cutoff and 2.0 ng/mL literature cutoff for severe sepsis (Harbarth et al., 2001). These values were significantly different from the sepsis value of 0.69 ng/mL but not different from the Neg SIRS and SIRS category values. In these groups, only 7 of 27 severe sepsis subjects and 3 of 16 septic shock subjects had SPCTC values less than the 1.62 ng/mL cutoff. As with the sepsis category, there is no obvious explanation for the decreased SPCTC values in the presence of an identified infection, except possibly the use of antibiotics. Only 5 of 27

المنارة للاستشارات
www.manaraa.com

severe sepsis subjects and 3 of 16 septic shock subjects did not receive antibiotics. Again, full bacterial culture reports were not available. The CRF did not contain information related to quantity of bacterial growth or antibiotic sensitivities that might have helped determine some of the differences among the various severity groups.

The SPCTC values of the ALF subjects when categorized by SIRS criteria appear to be similar to those obtained in the general population noted in the literature, as can be seen in Table 26. However, ALF Neg SIRS and SIRS values were higher than the literature values in all but one case (Giamarellos-Bourboulis), and might be a result of the severe liver damage, with or without bacterial infection. There have been some previous indications in the literature that PCT values may be increased in subjects with severe liver damage. Subjects with solid tumors that had metastasized to the liver had a higher mean PCT value than control subjects and those with non-metastasized solid tumors (Matzaraki et al., 2007). The sepsis and severe sepsis ALF results were similar to the literature reports while the septic shock results were lower than all but one of the literature reports (Brunkhorst et al., 2000; Castelli et al., 2004; E. J. Giamarellos-Bourboulis et al., 2002b; Harbarth et al., 2001; Oberhoffer, Vogetsang, Rubwurm, Hartung, & Reinhart, 1999).

In this study when SPCTC values were compared to AST and ALT values (indicative of the degree of liver damage) there were significant correlations. Spearman's rho analysis produced a correlation coefficient of 0.229 (df = 111, p-value = 0.015) for AST and 0.192 (df = 110, p-value = 0.042) for ALT supporting the idea that SPCTC may be increased in severe liver disease even in the absence of infection.

المنارة للاستشارات
www.manaraa.com

Table 26: Comparison of SPCTC Results by SIRS Category Between This Study and Previously Published Studies

	ALF	**Giamarellos-Bourboulis**	**Harbarth**	**Castelli**	**Brunkhorst**	**Oberhoffer**
	Mean / Median [ng/mL]	**Mean [ng/mL]**			**Median [ng/mL]**	
Neg SIRS / Not Infected	3.38 / 1.57	0.61	<0.2	0.14	na	na
SIRS	3.74 / 2.29	5.45	0.6	0.38	0.41	1.3
Sepsis	5.43 / 0.69	7.29	3.5	3.0	0.53	2.0
Severe Sepsis	11.99 / 3.46	6.26	6.2	5.58	6.91	8.7
Septic Shock	13.14 / 5.89	38.76	21.3	13.1	12.89	39

Missing data, might have led to false classification of subjects. Because the missing data were treated as negative values, subjects may have been classified in a severity category that was lower than the severity category they might have been classified in if all data were available.

Specific Aim Two

The purpose of this portion of the study was to determine the utility of SPCTC as a screening biomarker for infection in ALF and ALI patients.

H_0: There is no difference in SPCTC in ALF/ALI patients between the first day a positive culture was collected and the three days prior to a positive culture.

Serial samples from 34 subjects were examined for this purpose. The selection process for this set of samples prescribed that there was no documented infection prior to the day of infection, which was the day of collection of a culture that resulted in growth of a bacterial pathogen. However, when the data were examined, there was no significant difference in SPCTC results between the four days (d-3, d-2, d-1, and DoI) with a χ^2 = 6.741 (n = 34, df = 3, p-value = 0.081). There were individual cases for which the

المنارة للاستشارات
www.manaraa.com

SPCTC of the DoI increased over that of the D-3. Other cases within the category had higher SPCTC results on D-3 than those on DoI and other cases had low SPCTC results on D-3 that did not increase by DoI. When the subjects were sorted based upon the SPCTC from D3 and DoI, there is some indication as to possible reasons for the results that were obtained. The subjects were sorted based upon the following criteria:

Subgroup A samples, categorized based upon a D-3 level of > 3 ng/mL, had a decrease in the PCT values between D3 and DoI. As discussed earlier, there are several possible factors that may have affected the results. Subgroup A had the largest use of antibiotics (85.7%) of all of the subgroups (B = 50.0% and C = 75.0%) and antibiotics were more frequently used than for the whole group (73.5%). Based upon the literature (Christ-Crain et al., 2004; Schuetz et al., 2009), this may be an indication that there were unreported or unrecognized infections that antibiotics use were helpful in resolving. Again, there was no documentation of negative cultures to confirm a diagnosis of no infection and cultures were ordered at the treating physician's discretion. Also noted, the majority of the cases with the highest SPCTCs were from the acetaminophen toxicity etiology. It is uncertain what the physiological process in the acetaminophen toxicity patients is that might result in higher SPCTC results than in other ALF etiologies or if these subjects had underlying infectious processes that went unrecognized. The later would seem less likely because APAP subjects tend to do well. The possible link of APAP toxicity resulting in increased SPCTC discussed earlier, seems a more likely explanation and will have to be examined in future studies.

المنارة للاستشارات
www.manaraa.com

Subgroup B samples were categorized based upon a D-3 SPCTC level of <3 ng/mL and increased by DoI. Overall, there was an increase in the SPCTC median values from 1.96 ng/mL (D-3), 1.99 ng/mL (D-2), 2.09 ng/mL (D-1), to 5.34 ng/mL (DoI). While the D-3 median SPCTC was above the 1.62 ng/mL cutoff calculated earlier, the SPCTC increased to above the literature documented 2.0 ng/mL cutoff for severe sepsis (Harbarth et al., 2001) by D-2. The SPCTC increased further by D-1 and DoI. This group also had the lowest usage of antibiotics and only contained 25% APAP etiology. This small subset of cases, counter to the other results, would support the idea that SPCTC can be indicative of an infection even prior to DoI, similar to results seen in the general population (Guven et al., 2002; Brunkhorst et al., 1999; Luyt et al., 2005).

Data from Subgroup C, categorized by having a D-3 result of ≤1.62 ng/mL, the calculated cut-off value, and decreased or remained below the cut-off by DoI, was the least interpretable of the subgroups. While 75.0% of this group had been prescribed antibiotics possibly suppressing bacterial growth, their antibiotic use was not different from antibiotic use by the whole group (73.5%). Again, there was insufficient information concerning bacterial growth and antibiotic sensitivity and dose administration to fully understand the results obtained. There were no other striking features of the group with the exception, that over half of the group has ALF etiologies in the “other” category (i.e., anything other than acetaminophen toxicity and viral hepatitis). Again, it is unknown how much, if any, affect ALF etiology had on the SPCTC values.

المنارة للاستشارات
www.manaraa.com

Overall, data from this group of subjects did not support the use of SPCTC as a predictor of infection. However, it could be argued that SPCTC results for Subgroups A and B were indicative of infection. Subgroup A may have had undetected infections at or prior to D-3 and SPCTC predicted infections in Subgroup B at or before DoI. Future controlled studies will be needed to better understand how useful SPCTC is in detecting infection in ALF and ALI patients.

Specific Aim Three

The purpose of Specific Aim 3 was to determine if changes in the SPCTC of ALF and ALI patients with a confirmed bacterial infection on day one would predict the outcome of the patients – either TFS or DoT.

H_0: There is no difference in SPCTC between ALF/ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.

H_0: There is no difference in the changes in the serial SPCTCs between ALF/ALI patients with a bacterial infection/sepsis who have transplant free survival and those who die or receive a liver transplant.

Serial samples from 47 subjects, 26 with TFS and 21 with DoT outcomes, were tested to determine the SPCTCs. The largest difference between the two groups is that 73.1% of the TFS group had APAP etiology while 76.2% of the DoT group had etiologies other than APAP and viral hepatitis. This overall difference in etiologies fits with the outcomes based upon etiology in the literature (Lee & Seremba, 2008) in which APAP etiology has a survival rate of 58% to 64% compared to only about 20% to 25% survival for other

المنارة للاستشارات
www.manaraa.com

etiologies including drug-induced, autoimmune, and indeterminate ALF. Use of antibiotics was also slightly higher in the DoT group 85.7% compared to 69.2% for the TFS group. Despite these differences, when etiology (APAP vs. Other Etiologies) was examined as a potential covariate in the statistical model, it was not significant. Prophylactic antibiotic use alone and antibiotic therapy alone were not significant. Coma grade (1 – 2 vs. 3 – 4) was also examined, for significance to the model. The distribution of coma grades between the TFS and DoT groups was similar and there was no significance in the statistical model. While infection is associated with higher HEs, higher HEs are also associated with lower rates of spontaneous survival (Vaquero, 2003). The lack of difference between coma grade and the two groups may be attributed to the differences in etiology. While both groups have similar coma grade results, APAP has a better survival rate than other etiologies, even in the presence of higher coma grades and infection (Vaquero, 2003).

The only other differences noted, were an increase in the bilirubin and lactate values on DoI (Day 1) in the DoT group when compared to the TFS outcome group. Although there were no other significant differences in other indicators of liver function (i.e., AST, ALT, and INR), the bilirubin and lactate differences may be a function of the differences in etiologies or the severity of the liver disease.

The composite data from this portion of the study would suggest that there was little difference in SPCTC values between the two outcome groups. However, on closer examination, the median SPCTC values for the TFS group (which had a large percentage of APAP cases – 73.1%) were higher than the DoT group (76.2% etiologies other than

المنارة للاستشارات
www.manaraa.com

APAP) median SPCTC values. Both groups demonstrated elevated median values on Day 1, TFS = 2.90 ng/mL and DoT = 1.42 ng/mL, however, the median SPCTCs for the TFS survival group decreased faster than those for the DoT group. When the use of antibiotics was factored into the results, the difference between the two groups was even more apparent (see Figure 15 above). These results were similar to those reported in the literature by others (P. E. Charles et al., 2009) when looking to use of PCT to guide antibiotic therapy. In the PCT guided antibiotic therapy study, the decrease in SPCTCs in patients who received appropriate antibiotic therapy was larger than the decreases seen in patients who did not receive appropriate therapy. It cannot be determined from our data whether appropriate antibiotic therapy was used because the ALF CRFs did not include full microbiology reports with antibiotic sensitivities or specific information documenting when a specific antibiotic therapy was initially given. However, when the SPCTC profiles from the two studies are compared, the TFS group had a profile similar to the "appropriate use of antibiotics" profile, while the DoT profile was similar to that of subjects who received "inappropriate antibiotic therapy" (or those who did not respond to therapy). It would appear that ALF subjects with a documented infection who have a larger and faster decrease in SPCTC have a better outcome (i.e., TFS) than subjects whose SPCTC do not decease as much or as rapidly (DoT group). However, further study in which antibiotic use and etiology are controlled for will be required to confirm these findings.

المنارة للاستشارات
www.manaraa.com

Conclusions

Procalcitonin has proven to be useful in detecting and monitoring treatment for infection and sepsis in the general population. While the results of this study were hampered by factors that could not be totally controlled for or eliminated, there are some important conclusions that can be made. High SPCTC values in ALF subjects are not indicative of bacterial infection in many cases. While there were cases of high SPCTC values in the presence of infection, there were some subjects who had a high SPCTC but did not have a documented infection to justify the high SPCTC result. Further elucidation of the relationship between increased SPCTC and severe liver disease, particularly acute liver damage such as that resulting from APAP toxicity, is required. In addition, the SPCTC values do appear to fall in the presence of antibiotic therapy in patients who achieve transplant free survival but may also have simply been the result of the recovery process itself.

What was not answered clearly is 'What is the best cut-off value to indicate the presence or absence of a bacterial infection in this unique patient population?' The results of the study were confounded by the presence of antibiotics, missing data, and small sample sizes in some categories, despite the fact that this study utilized the largest collection of data and samples ever amassed for this rare condition. While a cutoff of 1.62 ng/mL was calculated for the ALF/ALI population, the sensitivity (the ability of a test to detect an infection in the presence of the infection) and specificity (the ability of a test to not detect an infection when an infection is not present), 64.3% and 62.0% respectively, were lower than those obtained in general population studies. When a ROC

المنارة للاستشارات
www.manaraa.com

analysis was performed on non-APAP results, the cut-off remained the same (1.62 ng/mL), but sensitivity decreased (54.3%) and specificity increased (69.2). Whether these changes reflect the differences in etiology or were a result of a smaller sample size, will have to be determined by later studies. In a study by (Harbarth et al., 2001), a 97% sensitivity and a 78% specificity were obtained at a cutoff of 1.1 ng/mL. Strong evidence was provided that etiology affects these results. The sample size of the various etiologies was too small for a clear determination of this effect. Further studies are needed to discern the true utility of procalcitonin adequately in the acute liver failure population.

Infection was not readily detected in all cases. While in a small subset of subjects infection was marked by an increased the SPCTC value on the DoI, other subjects had increased SPCTC values up to three days (this was the maximum amount of time studied) prior to the DoI while other subjects no elevated SPCTCs prior to or on the DoI. Again, this is an indication that SPCTC may not be useful in this population.

The one promising aspect of this study was the fact that SPCTC values over time were suggestive of outcome. SPCTC values that had a larger and faster decrease over time were indicative of TFS while, slower, smaller decreases were indicative of DoT.

Limitations of the Study

The use of retrospectively collected data and samples allowed for a relatively quick completion of this study despite the small target population, but it was also the greatest limitation to this study. The acute liver failure databases are prospectively collected studies designed to study acute liver failure not bacterial infection. The low incidence of acute liver failure in the US (approximately 2500 cases per year) makes

المنارة للاستشارات
www.manaraa.com

studying ALF challenging. The number of different etiologies confounds this even more. In addition, only about 30% of the ALF cases (based upon the number of documented infections in the USALFSG acute liver failure and acute liver injury databases) have a documented infection.

While this large retrospective database allowed for quick examination of many types of problems affecting patients with ALF, there were factors that had to be addressed. The ALF and ALI databases contain a large amount of missing data. The databases were prospectively collected observational studies. This means that the database and biosample repository were compiled from available data and samples. Unlike a clinical trial in which data and samples are collected at prescribed times, an observational study relies on available data. ALF subjects may not have certain labs or samples collected for a variety of reasons: labs not ordered, dis-enrolled because of liver transplant, death, and protocol changes over the course of the 10+ years of the ALF study changed what information was collected on the CRFs. Another reason for missing samples was that the supply of aliquots of samples from certain time-points was depleted.

All of these factors undoubtedly affected the results of this study. Missing data may have affected how samples were classified. The SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference included a long list of biodata parameters that can be used to classify patients along the SIRS continuum. While many of the ALF and ALI parameters recorded in the CRFs of ALF and ALI subjects are among those identified by the sepsis conference, many other parameters that could have been used were not routinely recorded. Of those that were routinely collected, some were not

المنارة للاستشارات
www.manaraa.com

collected on each day of the study. For this reason, subjects in the study were classified based upon available data. This meant that subjects may have been mis-classified. An example –a subject classified in the sepsis category should actually have been in the severe sepsis or septic shock category because laboratory parameters that would have changed the classification were missing. The best option would be not to use this subject, but a review of the available data showed that of the 12,492 patient days, for the 1863 subjects of this study, available for use in this study only 3% of the all days for all subjects had complete data. The study could not have been accomplished with only 3% of the database available. Therefore, to maximize the sample availability, use of subjects with missing data was allowed. In Specific Aim One, for the purpose of classifying subjects, missing values were treated as negative values.

Missing data did not change the status of samples in the SIRS and septic shock categories because samples in the categories were included only if specific criteria were met. Neither a positive nor a negative value for any missing data changed the result of a sample included in these two categories. However, missing data may have affected the sepsis and severe sepsis categories. If a sepsis sample had missing data that had a positive result, it could mean the sample should have been in the severe sepsis group (or severe sepsis should have been in the septic shock group). However, it is unclear whether the sepsis and severe sepsis groups can be clearly separated in the ALF population because by definition all septic ALF patients should have been considered in the severe sepsis category because of the liver failure. The attempt to separate these two groups was to show a progression of severity. By allowing samples with missing data to be categorized,

المنارة للاستشارات
www.manaraa.com

sample availability was maximized for categories with fewer available samples. The non-infected category was filled with subjects who are negative for SIRS and do not have a documented infection.

Limited availability of samples in the sample repository also affected all sections of this study. The sepsis and septic shock categories in the Specific Aim One section had less than half of the samples requested for the study. Part of the problem with these categories, was there were more subjects with available biodata than had available serum samples. This was also true for Specific Aims Two and Three. Specific Aim Two was limited to 34 subjects from a requested 45 due to limited availability of sample aliquots. Specific Aim Three was limited to 47 subjects from a requested 60.

Missing data and samples also affected how the results were analyzed. In Specific Aims One and Three, the analyses used were adjusted to account for missing data. In Specific Aim One, data related to etiology were not analyzed because in the cases of the sepsis and septic shock categories and the viral hepatitis group, there were not enough samples to provide an adequate statistical analysis. In the case of Specific Aim Three, a mixed methods analysis of covariance was used instead of a standard ANOVA and/or ANCOVA. The mixed methods approach allows for the missing data without losing degrees of freedom.

The ALF databases were not primarily designed to study infection. A prospective study designed to examine the use of SPCTC as a biomarker of infection, would control for antibiotic use, collect complete culture and sensitivity reports, and collect laboratory results relevant to correct classification to the subjects. This was not the case in the ALF

المنارة للاستشارات
www.manaraa.com

databases. While antibiotic use (both prophylaxis and therapy) was captured, dates and times of initiation of antibiotics were not captured. Also only minimal culture information was collected (i.e., culture type, day of the study the culture was collected, and organisms reported). No sensitivity information relevant to the pathologic organism was collected. Collection of cultures was based upon physician discretion and not under set conditions. Infection was determined as present in all parts of the study **only** if a positive culture was present. It was possible that an infection was present, in the population but was not found by standard culture techniques and therefore not documented. All of these factors affected the ability to interpret the results of this study correctly.

Another limitation of the use of retrospective samples from the ALF repositories was the age of the samples. There is evidence that long-term storage of samples results in a decrease in the SPCTC results. Schuetz, et al. (2010) studied two sets of samples that were store at -80° C for 4.8 to 5.5 years and 3.3 to 4.6 years. The SPCTCs for each set decrease by 11.4% and 13.5% respectively. ALF samples used in this study, were stored an average of approximately 6.75 years at -80° C storage. While it is assumed, that the length of time in storage has decreased the SPCTCs obtained, the exact amount of decrease is unknown and an estimate of the extent of the effect cannot be calculated. The assumption made for this study was that the affect is equal for all samples but in reality, this is probably not the case.

The use of the ALF databases addressed a potential threat to external validity. The databases collect samples from subjects enrolled in the studies from medical centers

المنارة للاستشارات
www.manaraa.com

across the US. The USALFSG has 13 active clinical sites. Prior to 2010, there were a maximum of 23 clinical centers in the study. All of the clinical sites are major medical/academic centers. Because smaller medical facilities are not included in this study, this could be considered a threat to external validity. However, due to the severity of illness presented by patients with ALF, many of these patients were transferred to larger facilities to take advantage of a possible liver transplant (to date - the only approved treatment for ALF other than supportive care) (Bower et al., 2007).

The one threat to external validity that has not been addressed by this study was the use of only one PCT testing platform and only one instrument on that platform. To date, FDA approval is limited in the US to the BRAHMS KRYPTOR and bioMérieux Clinical Diagnostics Vidas analyzers. These platforms were not available for use in this study. Siemens Healthcare Diagnostics has developed the ADVIA Centaur BRAHMS PCT assay that is currently in the FDA approval for use process but has not received final approval. The assay has been approved for use in the European Union. The ADVIA Centaur PCT assay has shown good comparison to the KRYPTOR PCT assay. Despite the fact that BRAHMS licenses all available assays, there is no standardization between the assays. Due to the limited sample volume for each serum aliquot, only one result for each sample was obtained and all samples for this study were tested on one analyzer. All of these factors allow for potential differences in results if this study were to be repeated on a different instrument or assay system.

www.manaraa.com

Recommendations for Future Studies

Despite the valuable information provided by this study, the best method to answer the questions left unanswered by this study would be a controlled clinical trial of prospectively enrolled subjects. A clinical trial with controlled conditions would diminish or eliminate the effect of many of the problems encountered in this study. A prospective, controlled clinical trial could:

- establish minimum sample sizes for all categories (i.e., severity of illness, etiology, outcome, etc.)
- control the use of antibiotics – which antibiotics were used, documentation of times of antibiotic initiation and discontinuation, availability of antibiotic sensitivities for the identified bacterial organism to determine appropriateness of prescribed antibiotics, and guidelines for antibiotic prophylaxis and therapy;
- screen for the presence or absence of bacterial infections;
- inclusion/exclusion criteria to provide a better categorization of subjects within the study;
- limit the amount of missing data – specific biomarkers and laboratory values would be collected for all subjects at all specified times; and
- limit missing samples – appropriate samples and sample volumes would be collected for all subjects for all required time points.

Procalcitonin has been described as a “homokine”, a protein that has some characteristics of a hormone and some characteristics of a cytokine. Cytokine results vary greatly depending upon disease and immune response. This study did not have enough

المنارة للاستشارات
www.manaraa.com

sample size to study this. Based upon the results of this study, a closer look at different ALF etiologies in relation to SPCTC values in the presence and absence of infection may prove useful. There was some indication in this study, that SPCTC results in subjects with acetaminophen toxicity may be different from the SPCTC results from other etiologies. A prospective study with a larger sample size in which determination of infection and antibiotic use in a comparison of acetaminophen toxicity vs. non-APAP ALF patients should be studied to adequately determine if APAP toxicity affects the procalcitonin results.

A prospective, controlled study could potentially identify the best SPCTC cut-off value(s) for identifying infection and sepsis in ALF patients (and if necessary specific populations within ALF such as APAP toxicity) which could speed the identification of bacterial infection and sepsis in this critically ill population. It could determine the utility of antibiotic guided therapy in ALF which could shorten exposure to hepatotoxic drugs and eliminate unnecessary prophylaxis. And, it would provide more information regarding the relationship between PCT and severe liver disease, particularly APAP toxicity.

المنارة للاستشارات
www.manaraa.com

REFERENCES

Advia centaur and advia centaur XP systems - procalcitonin (PCT) (2010). (Rev B ed.). Frimley, Camberley, UK: Siemens Healthcare Diagnostics, Ltd.

Advia centaur immunoassay system reference manual (2003). (078D0039-01 Rev. A ed.). Tarrytown, NY: Bayer Corporation.

Antoniades, C. G., Berry, P. A., Wendon, J. A., & Vergani, D. (2008). The importance of immune dysfunction in determining outcome in acute liver failure. *Journal of Hepatology, 49*, 845-861.

Aso, S., Baker, L., Freeman, J., & Navarro, D. (2009). Development of the ADVIA Centaur® BRAHMS procalcitonin (PCT) immunoassay for the risk assessment of critically ill patients in ICUs for progression to severe sepsis and septic shock [Abstract]. *Clinical Chemistry, 55* A147-Poster.

Assicot, M., Gendrel, D., Carson, H., Raymond, J., Guilbaud, J., & Bohuon, C. (1993). High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet, 341*(8844), 515-518.

Barrett, K. E. (2006). Functional anatomy of the liver and biliary system. *Gastrointestinal physiology.* The McGraw-Hill Companies, Inc.

Becker, K. L., Nylen, E. S., White, J. C., Muller, B., & Snider, R. H.,Jr. (2004). Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: A journey from calcitonin back to its precursors. *The Journal of Clinical Endocrinology and Metabolism, 89*(4), 1512-1525.

Bernal, W., Auzinger, G., Dhawan, A., & Wendon, J. (2010). Acute liver failure. *Lancet, 376*, 190-201.

Bernal, W., & Wendon, J. (2000). Acute liver failure. *Current Opinion in Anaesthesiology, 13*(2), 113-118. Boeker, K. H. W. (2001). Treatment of acute liver failure. *Metabolic Brain Disease, 16*(1/2), 103-117.

Bohuon, C. (2000). A brief history of procalcitonin. *Intensive Care Medicine, 26*, S146-S147.

المنارة للاستشارات
www.manaraa.com

Bota, D. P., Van Nuffelen, M., Zakariah, A. N., & Vincent, J. L. (2005). Serum levels of C-reactive protein and procalcitonin in critically ill patients with cirrhosis of the liver. *The Journal of Laboratory and Clinical Medicine, 146*(6), 347-351.

Bouadma, L., Luyt, C. E., Tubach, F., Cracco, C., Alvarez, A., Schwebel, C., et al. (2010). Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): A multicentre randomised controlled trial. *Lancet, 375*(9713), 463-474.

Bower, W. A., Johns, M., Margolis, H. S., Williams, I. T., & Bell, B. P. (2007). Population-based surveillance for acute liver failure. *The American Journal of Gastroenterology, 102*(11), 2459-2463.

Bracq, S., Machairas, M., Clement, B., Pidoux, E., Andreoletti, M., Mokhtar, M. S., et al. (1993). Calcitonin gene expression in normal human liver. *Federation of European Biochemical Societies Letters, 331*(1,2), 15-18.

BRAHMS PCT sensitive KRYPTOR (2008). (G. Hennigsdorf Trans.). BRAHMS Diagnostica, LLC.

Brun-Buisson, C. (2000). The epidemiology of the systemic inflammatory response. *SIntensive Care Medicine, 26*(Supp), S64-S74.

Brunkhorst, F. M., Eberhard, O. K., & Brunkhorst, R. (1999). Discrimination of infectious and noninfectious causes of early acute respiratory distress syndrome by procalcitonin. *Critical Care Medicine, 27*(10), 2172-2176.

Brunkhorst, F. M., Heinz, U., & Forycki, Z. F. (1998). Kinetics of procalcitonin in iatrogenic sepsis. *Intensive Care Medicine, 24*, 888-892.

Brunkhorst, F. M., Wegscheider, K., Forycki, Z. F., & Brunkhorst, R. (2000). Procalcitonin for early diagnosis and differentiation of SIRS, sepsis, severe sepsis, and septic shock. *Intensive Care Medicine, 26*, S148-S152.

Carrol, E. D., Thomson, A. P. J., & Hart, C. A. (2002). Procalcitonin as a marker of sepsis. *International Journal of Antimicrobial Agents, 20*, 1-9.

Castelli, G. P., Pognani, C., Meisner, M., Stuani, A., Bellomi, D., & Sgarbi, L. (2004). Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. *Critical Care (London, England), 8*(4), R234-42.

المنارة للاستشارات
www.manaraa.com

Charles, P. E., Tinel, C., Barbar, S., Aho, S., Prin, S., Doise, J. M., et al. (2009). Procalcitonin kinetics within the first days of sepsis: Relationship with the appropriateness of antibiotic therapy and the outcome. *Critical Care, 13*(2), 1-11.

Charles, P. E., Ladoire, S., Aho, S., Quenot, J. P., Doise, J. M., Prin, S., et al. (2008). Serum procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either gram negative or gram positive bacteria. *BMC Infectious Diseases, 8*(38), April 20, 2011-8.

Cheval, C., Timsit, J. F., Garrouste-Orgeas, M., Assicot, M., De Jonghe, B., Misset, B., et al. (2000). Protcalcitonin (PCT) is useful in predicting the bacterial origin of an acute circulatory failure in critically ill patients. *Intensive Care Medicine, 26*, S153-S158.

Christ-Crain, M., Jaccard-Stolz, D., Bingisser, R., Gencay, M. M., Huber, P. R., Tamm, M., et al. (2004). Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: Cluster-randomised, single-blinded intervention trial. *Lancet, 363*, 600-607.

Christ-Crain, M., & Muller, B. (2008). Calcitonin peptides -- the mediators in sepsis or just another fairy tale? *Critical Care Medicine, 36*(5), 1684-1687.

Clapperton, M., Rolando, N., Sandoval, L., Davies, E., & Williams, R. (1997). Neutrophil superoxide and hydrogen peroxide production in patients with acute liver failure. *European Journal of Clinical Investigation, 27*, 164-168.

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences, revised edition.* New York, NY: Academic Press.

Connert, S., Stremmel, W., & Eising, C. (2003). Procalcitonin is a valid marker of infection in decompensated cirrhosis. [Abstract]. *Z Gastroenerology, 41*(2) 165-170.

Dandona, P., Nix, D., Wilson, M. F., Aljada, A., Love, J., Assicot, M., et al. (1998). Procalcitonin increase after endotoxin injection in normal subjects. *The Journal of Clinical Endocrinology & Metabolism, 79*, 1605-1608.

DeLeve, L. D. (2003). Vascular diseases of the liver. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 2517-2525). Philadelphia, PA: Lippincott Williams & Wilkins.

Domenech, V. S., Nylen, E. S., White, J. C., Snider, R. H., Jr., Becker, K. L., Landmann, R, et al. (2001). Calcitonin gene-related peptide expression in sepsis : Postulation of

المنارة للاستشارات

www.manaraa.com

microbial infection-specific response elements within the calcitonin I gene promoter. *Journal of Investigative Medicine, 49*(6), 514-521.

Dornbusch, H. J., Strenger, V., Kerbl, R., Lackner, H., Schwinger, W., Sovinz, P., et al. (2005). Procalcitonin - a marker of invasive fungal infection? *Support.Care Cancer, 13*, 343-346.

Dragomir, A. -C., Laskin, J. D., Laskin, D. L. (2011). Macrophage activation by factors released from acetaminophen-injured hepatocytes: Potential role of HMGB-1. *Toxicology and Applied Pharmacology, 253*, 170-177.

du Plessis, M., Smith, A. M., & Klugman, K. P. (1998). Rapid detection of penicillin-resistant streptococcus pneumoniae in cerebrospinal fluid by a seminested-PCR strategy. *Journal of Clinical Microbiology, 36*(2), 453-457.

Dubos, F., Korczowski, B., Aygun, D. A., Martinot, A., Prat, C., Galetto-Lacour, A., et al. (2008). Serum procalcitonin level and other biological markers to distinguish between bacterial and aseptic meningitis in children - A european multicenter case cohort study. *Archives of Pediatric and Adolescent Medicine, 162*(12), 1157-1163.

Edwards, J. D. (1991). Oxygen transport in cardiogenic and septic shock. *Critical Care Medicine, 19*(5), 658-663.

Elefsiniotis, I. S., Petrocheilou, A., Scarmeas, N., Ketikoglou, I., Pantazis, K. D., Toutouza, M., et al. (2006). Serum procalcitonin levels in chronic hepatitis C patients under pegylated interferon-alpha plus ribavirin treatment. *Journal of Clinical Virology : The Official Publication of the Pan American Society for Clinical Virology, 37*(4), 329-331.

Elefsiniotis, I. S., Skounakis, M., Vezali, E., Pantazis, K. D., Petrocheilou, A., Pirounaki, M., et al. (2006). Clinical significance of serum procalcitonin levels in patients with acute or chronic liver disease. *European Journal of Gastroenterology & Hepatology, 18*(5), 525-530.

Elliot, A. C., & Hynan, L. S. (2011). A SAS® macro implementation of a multiple comparison post hoc test for a Kruskal-Wallis analysis. *Computer Methods and Programs in Biomedicine*, *102(1)*, 75-80.

Falcoz, P.-E., Laluc, F., Toubin, M.-M., Puyraveau, M., Clement, F., Mercier, M., et al. (2005). Usefulness of procalcitonin in the early detection of infection after thoracic surgery. *The European Journal of Cardio-Thoracic Surgery, 27*, 1074-1078.

المنارة للاستشارات
www.manaraa.com

Fenollar, F., & Raoult, D. (2007). Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. *International Journal of Antimicrobial Agents, 30S*, S7-S15.

Ferenci, P., Lockwood, A., Mullen, K., Tarter, R., Weissenbom, K., Blei, A. T., et al. (2002). Hepatic encephalopathy - definition, nomenclature, diagnosis, and quantification: Final report of the working party at the 11th world congresses of gastroenterology, vienna, 1998. *Hepatology, 35*(3), 716-721.

Francois, P., Pittet, D., Bento, M., Pepey, B., Vaudaux, P., Lew, D., et al. (2003). Rapid detection of methicillin-resistant staphylococcus aureus directly from sterile or nonsterile clinical samples by a new molecular assay. *Journal of Clinical Microbiology, 41*(1), 254-260.

Gebert, S., Siegel, D., & Wellinghauser, N. (2008). Rapid detection of pathogens in blood culture bottles by real-time PCR in conjunction with the pre-analytic tool MolYsis. *Journal of Infection, 57*, 307-316.

Geha, D. J., Uhl, J. R., Gustaferro, C. A., & Persing, D. H. (1994). Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. *Journal of Clinical Microbiology, 32*(7), 1768-1772.

Georghiou, P. R., Hamill, R. J., Wright, C. E., Versalovic, J., Koeuth, T., Watson, D. A., et al. (1995). Molecular epidemiology of infections due to enterobacter aerogenes: Identification of hospital outbreak-associated strains by molecular techniques. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 20*(1), 84-94.

Giamarellos-Bourboulis, E. J., Mega, A., Grecka, P., Scarpa, N., Koratzanis, G., Thomopoulos, G., et al. (2002a). Procalcitonin: A marker to clearly differentiate systemic inflammatory response syndrome and sepsis in the critically ill patient? *1351Intensive Care Medicine, 28*, 1351-1356.

Giamarellos-Bourboulis, E. J., Mega, A., Grecka, P., Scarpa, N., Koratzanis, G., Thomopoulos, G., et al. (2002b). Procalcitonin: A marker to clearly differentiate systemic inflammatory response syndrome and sepsis in the critically ill patient? *Intensive Care Medicine, 28*(9), 1351-1356.

Guven, H., Altintiop, L., Baydin, A., Esen, S., Aygun, D., Hokelek, M., et al. (2002). Diagnostic value of procalcitonin levels as an early indicator of sepsis. *American Journal of Emergency Medicine, 20*, 202-206.

المنارة للاستشارات
www.manaraa.com

Harbarth, S., Holeckova, K., Froidevaux, C., Pittet, D., Ricou, B., Grau, G. E., et al. (2001). Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. *American Journal of Respiratory Critical Care Medicine, 164*, 396-402.

Heathcote, E. J. (2003). Autoimmune hepatitis. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 2366-2374). Philadelphia, PA: Lippincott Williams & Wilkins.

Ho, Y.-P., & Reddy, P. M. (2010). Identification of pathogens by mass spectrometry. *Clinical Chemistry, 56*(4), 525-536.

Hsueh, P. R., Teng, L. J., Pan, H. J., Chen, Y. C., Sun, C. C., Ho, S. W., et al. (1998). Outbreak of pseudomonas fluorescens bacteremia among oncology patients. *Journal of Clinical Microbiology, 36*(10), 2914-2917.

IBM SPSS Statistics Software, Version 19, (2010), SPSS, Inc., Chicago, Illinois. Software retrieved through Virginia Commonwealth University, Richmond, VA.

Immunoluminometric assay (ILMA) for the determination of PCT (procalcitonin) in human serum and plasma (coated tube system) (2005). (G. Hennigsdorf Trans.). BRAHMS Diagnostica, LLC.

Jaeschke, H., McGill, M. R., Ramachandran, A. (2012). Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. *Drug Metabolism Reviews, 44*(1), 88-106.

Jaffe, R. I., Lane, J. D., Albury, S. V., & Niemeyer, D. M. (2000). Rapid extraction from and direct identification in clinical samples of methicillin-resistant staphylococci using the PCR. *Journal of Clinical Microbiology, 38*(9), 3407-3412.

Jin, M., & Khan, A. I. (2010). Procalcitonin: Uses in the clinical laboratory for the diagnosis of sepsis. *Laboratory Medicine, 41*(3), 173-177.

Khalili, M., Liao, C. E., & Nquyen, T. (2010). Chapter 14. liver disease. In S. J. McPhee, & G. D. Hammer (Eds.), *Pathophysiology of disease: An introduction to clinical medicine* (6th ed.,) The McGraw-Hill Companies, Inc.

Khashab, M., Tector, A. J., & Kwo, P. Y. (2007). Epidemiology of acute liver failure. *Current Gastroenterology Reports, 9*(1), 66-73.

Kilic, A., Muldrew, K. L., Tang, Y., & Basustaoglu, A. C. (2010). Triplex real-time polymerase chain reaction assay for simultaneous detection of

المنارة للاستشارات

www.manaraa.com

staphylococcus aureus and coagulase-negative staphylococci and determination of methicillin resistance directly from positive blood culture bottles. *Diagnostic Microbiology and Infectious Disease, 66*, 349-355.

Kollef, M. H. (2010). Will procalcitonin reduce antibiotic use in intensive care? *Lancet, 375*(9713), 435-436.

Lee, W. M. (2003). Acute liver failure in the united states. *Seminars in Liver Disease, 23*(3), 217-226.

Lee, W. M., & Seremba, E. (2008). Etiologies of acute liver failure. *Current Opinion in Critical Care, 14*, 198-201.

Lee, W. M., Squires, J., R.H., Nyberg, S. L., Doo, E., & Hoofnagle, J. H. (2008). Acute liver failure: Summary of a workshop. *Hepatology, 47*, 1401-1415.

Levinson, W. (2010). Chapter 9. laboratory diagnosis. *Levinson W: Review of medical microbiology and immunology*. Retrieved from 10e: http://www.accessmedicine.com.foyer.swmed.edu/content.aspx?aID=3339868.

Levy, M. M., Fink, M. P., Marshall, J. C., Abraham, E., Angus, D., Cook, D., et al. (2003). 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Critical Care Medicine, 31*(4), 1250-1256.

Luyt, C., Guerin, V., Combes, A., Trouillet, J., Ayed, S. B., Bernard, M., et al. (2005). Procalcitonin kinetics as a prognostic marker of ventilator-associated pneumonia. *American Journal of Respiratory and Critical Care Medicine, 171*, 48-53.

Mallinckrodt, C. H., Kaiser, C. J., Watkin, J. G., Molenberghs, G., Carroll, R. J. (2004). The effect of correlation structure on treatment contrasts estimated from incomplete clinical trial data with likelihood-based repeated measures compared with last observation carried forward ANOVA. *Clinical Trials, 1,* 477-489.

Martini, A., Gottin, L., Menestrina, N., Schweiger, V., Dimion, D., & Vincent, J. (2010). Procalcitonin levels in surgical patients at risk of candidemia. *Journal of Infection, 60*, 425-430.

Matzaraki, V., Alexandraki, K. I., Venetsanou, K., Piperi, C., Myrianthefs, P., Malamos, N., et al. (2007). Evaluation of serum procalcitonin and interleukin-6 levels as markers of liver metastasis. *Clinical Biochemistry, 40*, 336-342.

المنارة للاستشارات

www.manaraa.com

McGee, K. A., & Baumann, N. A. (2009). Procalcitonin: Clinical utility in diagnosing sepsis. *Clinical Laboratory News, 35*(7), 10-12.

Meisner, M., Huettemann, L. E., Schmidt, J., Hueller, M., & Reinhart, K. (2001). The plasma elimination rate and urinary secretion of procalcitonin in patients with normal and impaired renal function. *European Journal of Anaesthesiology, 18*, 79-87.

Meisner, M., Schmidt, J., Huttner, H., & Tschaikowsky, K. (2000). The natural elimination rate of procalcitonin in patients with normal and impaired renal function. *Intensive Care Medicine, 26*(Suppl.), 212-216.

Meisner, M., Tschaikowsky, K., Hutzler, A., Schick, C., & Schuttler, J. (1998). Postoperative plasma concentrations of procalcitonin after different types of surgery. *Intensive Care Medicine, 24*, 680-684.

Mendler, M., Donovan, J., & Blei, A. (2003). Central nervous system and pulmonary complications of end-stage liver diseases. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 2445-2468). Philadelphia, PA: Lippincott Williams & Wilkins.

Merriman, R. B., & Peters, M. G. (2003). Approach to the patient with jaundice. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 911-928). Philadelphia, PA: Lippincott Williams & Wilkins.

Mindikoglu, A. L., Magder, L. S., & Regev, A. (2009). Outcome of liver transplantation for drug-induced acute liver failure in the united states: Analysis of the united network for organ sharing database. *Liver Transplantation : Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society, 15*(7), 719-729.

Mohrman, D. E., & Heller, L. J. (2006). Chapter 3. the heart pump. *Cardiovascular physiology* (6th ed.,) The McGraw-Hill Companies, Inc.

Moseley, R. H. (2003). Approach to the patient with abnormal liver chemistries. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 929-947). Philadelphia, PA: Lippincott Williams & Wilkins.

Muller, B., & Becker, K. L. (2001). Procalcitonin: How a hormone became a marker and mediator of sepsis. *Swiss Medical Weekly : Official Journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine, the Swiss Society of Pneumology, 131*(41-42), 595-602.

المنارة للاستشارات

www.manaraa.com

Muller, B., Christ-Crain, M., & Schuetz, P. (2007). Meta-analysis of procalcitonin for sepsis detection. *The Lancet Infectious Diseases, 7*(8), 498-9; author reply 502-3.

Murray, K. F., Hadzic, N., Wirth, S., Bassett, M., & Kelly, D. (2008). Drug-related hepatotoxicity and acute liver failure. *Journal of Pediatric Gastroenterology and Nutrition, 47*(4), 395-405.

Nakamura, A., Wada, S., Takeda, T., & Nobori, T. (2009). Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. *Shock, 31*(6), 586-591.

Nylen, E. S., Whang, K. T., Snider, R. H., Jr., Steinwald, P. M., White, J. C., & Becker, K. L. (1998). Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. *Critical Care Medicine, 26*(6), 977-978.

Nystrom, P. (1998). The systemic inflammatory response syndrome: Definitions and aetiology. *Journal of Antimicrobial Chemotherapy, 41*(Supplement A), 1-7.

Oberhoffer, M., Vogetsang, H., Rubwurm, S., Hartung, T., & Reinhart, K. (1999). Outcome prediction by traditional and new markers of inflammation in patients with sepsis. *Clinical Chemistry and Laboratory Medicine, 37*(3), 363-368.

O'Grady, N. P., Barie, P. S., Bartlett, J. G., Bleck, T., Carroll, K., Kalil, A. C., et al. (2008). Guidelines for evaluation of new fever in critically ill adult patients: 2008 update from the american college of critical care medicine and the infectious diseases society of america. *Critical Care Medicine, 36*, 1330-1349.

Ostapowicz, G., Fontana, R. J., Schiodt, F. V., Larson, A., Davern, T. J., Han, S. H. B., et al. (2002). Results of a prospective study of acute liver failure at 17 tertiary care centers in the united states. *Annals of Internal Medicine, 137*(12), 947-954.

PCT and its role in identifying and monitoring patients with sepsis. (n.d.). Retrieved August 4, 2010, from http://www.procalcitonin-testing.com/aboutPCT.html

Peters, R. P., van Agtmael, M. A., Danner, S. A., Savelkoul, P. H., & Vandenbroucke-Grauls, C. M. (2004). New developments in the diagnosis of bloodstream infections. *The Lancet Infectious Diseases, 4*(12), 751-760.

Polit, D. F., & Beck, C. T. (2008). In Surrena H., Kogut H. (Eds.), *Nursing research: Generating and assessing evidence for nursing practice* (8th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

المنارة للاستشارات
www.manaraa.com

Polson, J., & Lee, W. M. (2007). Etiologies of acute liver failure: Location, location, location. *Liver Transplantation, 13*, 1362-1363.

Questions and answers for transplant candidates about MELD AND PELD. (2008). Retrieved January 12, 2011, from http://www.unos.org/docs/MELD_PELD.pdf

Rangel-Frausto, M. S. (2005). Sepsis: Still going strong. *Archives of Medical Research, 36*, 672-681.

Rangel-Frausto, M. S., Pittet, D., Costigan, M., Hwang, T., Davis, C. S., & Wenzel, R. P. (1995). The natural history of the systemic inflammatory response syndrome (SIRS): A prospective study. *Journal of the American Medical Association, 273*(2), 117-123.

Reith, H. B., Mittelkotter, U., Wagner, R., & Thiede, A. (2000). Procalcitonin (PCT) in patients with abdominal sepsis. *Intensive Care Medicine, 26*, S165-S169.

Riffenburgh, R. H. (2006). *Statistics in medicine* (2nd ed.). New York, NY: Elsevier.

Rolando, N., Harvey, F., Brahm, J., Philpott-Howard, J., Alexander, G., Gimson, A., et al. (1990). Prospective study of bacterial infection in acute liver failure: An analysis of fifty patients. *Hepatology, 11*(1), 49-53.

Rolando, N., Wade, J., Davalos, M., Wendon, J., Philpott-Howard, J., & Williams, R. (2000). The systemic inflammatory response syndrome in acute liver failure. *Hepatology, 32*(4), 734-739.

Ruimy, R., Dos-Santos, M., Raskine, L., Bert, F., Masson, R., Elbaz, S., et al. (2008). Accuracy and potential usefulness of triplex real-time PCR for improving antibiotic treatment of patients with blood cultures showing clustered gram-positive cocci on direct smears. *Journal of Clinical Microbiology, 46*(6), 2045-2051.

Russwurm, S., Oberhoffer, M., Zipfelk, P. F., & Reinhart, K. (1999). Procalcitonin - A novel biochemical marker for the mediator-directed therapy of sepsis. *Molecular Medicine Today, 5*, 286-287.

Sabet, N. S., Subramaniam, G., Navaratnam, P., & Sekaran, S. D. (2006). Simultaneous species identification and detection of methicillin resistance in staphylococci using triplex real-time PCR assay. *Diagnostic Microbiology and Infectious Disease, 56*(1), 13-18.

المنارة للاستشارات
www.manaraa.com

Sarwari, A., Hasan, R., Lim, C. B., Ng, Y., Ng, C., & Zaman, S. (2004). PCR identification and automated ribotyping of pseudomonas aeruginosa clinical isolates from intensive care patients. *Scandinavian Journal of Infectious Diseases, 36*(5), 342-349.

SAS Statistics Software, Version 9.2, (2008), SAS Institute Inc., Cary, North Carolina.

Schiodt, F. V., Atillasoy, E., Shakil, A. O., Schiff, E. R., Caldwell, C., Kowdley, K. V., et al. (1999). Etiology and outcome for 295 patients with acute liver failure in the united states. *Liver Transplatation and Surgery, 5*(1), 29-34.

Schrenzel, J. (2007). Clinical relevance of new diagnostic methods for bloodstream infections. *International Journal of Antimicrobial Agents, 30S*, S2-S6.

Schuetz, P., Christ-Crain, M., Huber, A. R., & Muller, B. (2010). Long-term stability of procalcitonin in frozen samples and comparison of kryptor and VIDAS automated immunoassays. *Clinical Biochemistry, 43*(3), 341-344.

Schuetz, P., Christ-Crain, M., & Muller, B. (2009). Procalcitonin and other biomarkers to improve assessment and antibiotic stewardship in infections - hope for hype? *Swiss Medical Weekly : Official Journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine, the Swiss Society of Pneumology, 139*(23-24), 318-326.

Schuetz, P., Christ-Crain, M., Thomann, R., Falconnier, C., Wolbers, M., Widmer, I., et al. (2009). Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: The ProHOSP randomized controlled trial. *Journal of the American Medical Association, 302*(10), 1059-1066.

Sixou, M. (2003). Diagnostic testing as a supportive measure of treatment strategy. *Oral Diseases, 9 Suppl 1*, 54-62.

Sokol, R. J. (2003). Metabolic diseases of the liver. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 2397-2414). Philadelphia, PA: Lippincott Williams & Wilkins.

Spahr, L., Morard, I., Hadengue, A., Vadas, L., & Pugin, J. (2001). Procalcitonin is not an accurate marker of spontaneous bacterial peritonitis in patients with cirrhosis. *Hepatogastroenterology, 48*(38), 502-505.

Stefani, S. (2009). Diagnostic techniques in bloodstream infections: Where are we going? *International Journal of Antimicrobial Agents, 34S*, S9-S12.

المنارة للاستشارات
www.manaraa.com

Steinbach, G., Rau, B., Debard, A. -., Javourez, J. -., Bienvenu, J., Ponzio, A., et al. (2004). Multicenter evaluation of a new immunoassay for procalcitonin measurement on the kryptor system. *Clinical Chemistry and Laboratory Medicine, 42*(4), 440-449.

Stravitz, R. T. (2008). Critical management decisions in patients with acute liver failure. *Chest, 134*, 1092-1102.

Strommenger, B., Schmidt, C., Werner, G., Roessle-Lorch, B., Bachmann, T. T., & Witte, W. (2007). DNA microarray for the detection of therapeutically relevant antibiotic resistance determinants in clinical isolates of staphylococcus aureus. *Molecular and Cellular Probes, 21*(3), 161-170.

Suljagic, V., Cobeljic, M., Jankovic, S., Mirovic, V., Markovid-Denic, L., Romic, P., et al. (2005). Nosocomial bloodstream infections in ICU and non-ICU patients. *American Journal of Infection Control, 33*, 333-340.

Tang, H., Huang, T., Jing, J., Shen, H., & Cui, W. (2009). Effect of procalcitonin-guided treatment in patients with infections: A systemic review and meta-analysis. *Infection, 37*(6), 497-507.

Tissari, P., Zumla, A., Tarkka, E., Mero, S., Savolainen, L., Vaara, M., et al. (2010). Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: An observational study. *Lancet, 375*, 224-230.

Trey, C., & Davidson, C. S. (1970). The managment of fulminant hepatic failure. In H. Popper, & F. Schaffner (Eds.), *Progress in liver diseases* (pp. 282-298). New York, NY: Grune & Stratton.

United States Acute Liver Failure Study Group. (2011). *Adult ALI - outcome by etiology.* Unpublished manuscript.

Vaquero, J., Polson, J., Chung, C., Helenowski, I., Schiodt, F. V., Reisch, J., et al. (2003). Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology, 125*(3), 755-764.

Viallon, A., Zeni, F., Pouzet, V., Lambert, C., Quenet, S., Tardy, B., et al. (2000). Serum and ascitic procalcitonin levels in cirrhotic patients with spontaneous bacterial peritonitis: Diagnostic value and relationship to pro-inflammatory cytokines. *Intensive Care Medicine, 26*, 1082-1086.

المنارة للاستشارات

www.manaraa.com

Wade, J., Rolando, N., Philpott-Howard, J., & Wendon, J. (2003). Timing and aetiology of bacterial infections in a liver intensive care unit. *The Journal of Hospital Infection, 53*(2), 144-146.

Whang, K. T., Vath, S. D., Nylen, E. S., Muller, B., Li, Q., Tamarkin, L., et al. (1999). Procalcitonin and proinflammatory cytokine interactions in sepsis. *Shock, 12*(4), 268-273.

Zimmermann, E. M., & Christman, G. M. (2003). Approach to gastrointestinal and liver diseases in the female patient. In T. Yamada (Ed.), *Textbook of gastroenterology* (4th ed., pp. 1033-2055). Philadelphia, PA: Lippincott Williams & Wilkins.

المنارة للاستشارات

www.manaraa.com

APPENDIX A

Algorithms to Determine SIRS Category for Specific Aim One Samples

المنارة للاستشارات

www.manaraa.com

Appendix A: Algorithms to determine SIRS category for Specific Aim One samples

1 = Yes 0 = No

SIRS - If column I has a value of 1, the sample is categorized as positive for SIRS.

	A	B	C	D	E	F	G	H	I
	Temp <36	>38	Sum	Temp	pCO2 <32	WBC >12	<4		SIRS
	IF A<36=1 otherwise =0	IF A>38=1 otherwise =0	A + B	IF C=1, then 1 otherwise =0	IF E<32=1 otherwise =0	IF F>12=1 otherwise =0	IF G<4=1 otherwise =0	D+E+ F+G	IF H>1, then 1 otherwise =0
Sample#	0	0	0	0	0	1	0	1	0

Sepsis - If column L has a value of 1, the sample is categorized as positive for Sepsis.

	J	K	L
	Infection	Infection + SIRS	SEPSIS
	Infection =1, No infection =0	I + J	IF K>1, then 1 otherwise =0
Sample#	1	1	1

المنارة للاستشارات
www.manaraa.com

Severe Sepsis - If column AF has a value of 1, the sample is categorized as positive for Severe Sepsis. The group doesn't include patients with only liver failure in the group for septic shock. Patients with only liver failure will be included in the sepsis category.

	M	N	O	P	Q	R	S
	pO2/FiO2		Creatinine - previous Creatinine	>0.5	Acute ARF Adm	Renal Dialysis	Failure ARF
		IF M<300=1 otherwise =0		IF O<0.5=1 otherwise =0			IF P, Q, OR R >0 =1, otherwise =0
Sample#	281	1	-0.50	0	0	0	0

	T	U	V	W	X	Y	Z
	INR >1.5	Platelet <100,000	Bilirubin >4	Lactate >1	MAP	<70	HE / CG
	IF T>1.5=1 otherwise =0	IF U<100=1 otherwise =0	IF V>4=1 otherwise =0	IF W>1=1 otherwise =0		IF Y<70=1 otherwise =0	
Sample#	1	0	1	0	71	0	1

المنارة للاستشارات
www.manaraa.com

	AA	AB	AC	AD	AE	AF	AG
	with liver components N+S+T+U+ V+W+Y+Z		Severe Sepsis w Liver	without liver components N+S+U+ W+Y		Severe Sepsis wo Liver	Compare AC to AF
		IF AA>0, then 1 otherwise =0	IF L+AB=2, =1, otherwise =0		IF AD>0, then 1 otherwise =0	IF L+AE=2, =1, otherwise =0	IF AC=AF, =1, otherwise =0
Sample#	4	1	1	1	1	1	1

Septic Shock - If column AK has a value of 1, the sample is categorized as positive for Septic Shock.

	AH	AI	AJ	AK
	D1 MAP <60		Pressor therapy	Septic Shock map+prss+ sepsis
		IF AH<60=1 otherwise =0		IF L+AI+AJ=3, =1, otherwise =0
Sample#	55	1	1	1

المنارة للاستشارات
www.manaraa.com

VITA

Jody Anne Balko was born January 19, 1957, in Fort Polk, Louisiana and she is an American Citizen. She graduated from Vidalia High School in Vidalia, Georgia in 1975. She received her Bachelor of Science in Medical Technology from Georgia Southern College, Statesboro, Georgia in 1979. She received a Master of Medical Science in Clinical Chemistry from Emory University, Atlanta, Georgia in 1992.

Jody has worked as the laboratory supervisor of the Special Chemistry and Clinical Research Laboratory in the Department of Pathology at University of Texas Southwestern Medical Center. She currently is currently employed as a Clinical Research Manager in the Clinical Center for Liver Disease in the Department of Internal Medicine at the University of Texas Southwestern Medical Center.

المنارة للاستشارات

www.manaraa.com