Platelet Closure Time in Severe Sepsis and Shock

Major Qualifying Project

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1. 0 Introduction

The purpose of this project is to establish an early detection marker for patients with sepsis or septic shock. The current protocol to diagnose sepsis results in late detection. Lactate is currently used as a primary marker and once levels are higher than 4millimol/L, the patient is said to have sepsis. There are not defined levels for the stages of sepsis. In many cases the patient symptoms worsen quickly and invasive procedures need to be utilized for treatment. Further research into early goal directed therapy as a solution for early detection has the potential to give the patients better treatment prior to the development of severe sepsis or septic shock.

1.1 MQP Objectives

The hypothesis of the project is that platelet closure time will aid in early detection of sepsis. The first goal to validate the correlation between platelet closure time (PCT) and sepsis will be to analyze the PCT in 50 patients that fit the study criteria. The subsequent goal is to establish a correlation between lactate as biomarker and PCT.

2.0 Literature Background

2.1 Statistics

Sepsis is currently the leading cause of death in intensive care units as well as the eleventh cause of death in the United States. However, sepsis does not receive the research funding or publicity that other diseases such as acquired immune deficiency syndrome (AIDS), cardiac failure, and various cancers receive (Offord 2002). Approximately 750,000 cases of sepsis are diagnosed each year. The average cost of treating one sepsis case is $22,100, which totals 16.7 billion dollars annually in the United States (Offord 2002). The high mortality rate of sepsis results in approximately 215,000 deaths of the 750,000 cases. This is an extremely high rate for a disease that has such little public awareness and research funding (Offord, 2002). Due to the lack of funding there is still no definitive early biomarker that indicates a patient is about to become septic or go into septic shock. Currently, Emergency Room physicians rely on lactate levels as a definitive marker. When a lactate level is 4 millimol/L, it is clear that invasive goal-directed therapy is needed. However, when a lactate level is higher than normal, such as a 2 millimol/L it is unclear how to treat the patient. If there was an additional indicator that confirmed the patients' status, treatment could be started early with a potentially more successful outcome.

2.2 Sepsis

Sepsis is a systemic disease caused by a bacterial infection. The systemic infection leads to a body's natural response in release of inflammatory mediators. An increase in cardiac output is the body's first heightened immune response. A patient is diagnosed with sepsis when their initial symptoms are fever, tachypnoea (rapid, shallow breathing), and tachycardia (increased heart rate) (Offord,2002). There are a variety of microbial pathogens that can lead to the symptoms that classify a patient as septic.

2.2.1 Symptoms and Signs

There are several stages of sepsis that range from systemic inflammatory response syndrome (SIRS) to septic shock. The progression of each stage is displayed below in Table 1.

Table 1: ACCP/SCCM Consensus Definition for Sepsis and Organ Failure (Offord, 2002).

Table 1: ACCP/SCCM consensus definitions for sepsis and organ failure[1]

Terminology	Definition
Infection	Inflammatory response to micro-organisms Invasion of normally sterile tissues
Systemic inflammatory response syndrome (SIRS)	Clinical response arising from a non-specific insult, including more than two of the following: 1 Temperature 38C or 36C 1 Tachycardia; heart rate 90 beats per minute 1 Tachypnoea; respiratory rate 20 /minute White blood cell count 12,000 per mm^3 or 4,000 per mm^3 or >10 per cent immature neutrophils
Sepsis	SIRS with a presumed or confirmed infectious process
Severe sepsis	Sepsis with more than one sign of acute organ dysfunction: 1 Renal 1 Respiratory 1 Hepatic 1 Haematological 1 Central nervous system 1 Unexplained metabolic acidosis
Septic shock	Severe sepsis with cardiovascular dysfunction, characterised by hypotension refractory to adequate volume resuscitation
Multiple organ dysfunction syndrome (MODS)	Altered organ function in an acutely ill patient Inability to maintain haemostasis without intervention

The infections are commonly caused by gram-negative bacteria, which can originate in the lungs, abdomen, or urinary tract. Most cases seen are pulmonary infections. Upon entry into the Emergency Room if the patient appears to have two or more systemic inflammatory response syndrome (SIRS) symptoms (as listed in Table 1) along with a confirmed infection the patient is diagnosed as septic. In order for sepsis to progress to severe sepsis there must be signs of acute organ dysfunction. Severe sepsis accompanied by cardiovascular dysfunction and low blood pressure, is categorized as septic shock. An early detection marker could be crucial allowing physicians to start treatment before the patient enters the intensive care unit (ICU), as this disease can progress at a rapid pace (Offord, 2002).

In order to make early detection feasible it is important to study the factors that lead to organ failure in sepsis patients. This study is focusing on identifying the connection between the systemic inflammatory cascade and the coagulation cascade. If a better understanding is obtained, there may possibly be an early coagulation biomarker that could be used rather than relying on a systemic inflammatory marker such as lactate levels as in the current practice.

2.2.2 Early Goal Directed Therapy in Sepsis Patients

There has been an increase in successful outcomes of conditions such as stroke and acute myocardial infarctions due to early diagnosis and time-sensitive therapy at the proximal stage. This same practice applied to sepsis could potentially lead to improvements in the outcome of the patients. Early goal directed therapy (EGDT) is a complex strategy (Otero, pp. ,2006). Often therapy initiatives are started once the patient is transferred to the ICU. However, if early-goal directed therapy can help prevent sudden cardiovascular collapse then the need for vasopressors, mechanical ventilation, and pulmonary-artery catheterization decreases. Global tissue hypoxia is determined by a high lactate concentration. Hypoxia also stimulates systemic inflammatory response syndrome (SIRS). Early goal directed therapy does have its risks. It involves putting a central venous catheter into the patient so that the central venous oxygen saturation, the amount of oxygen saturation in the vena cava of the heart ($ScV0_2$), can be monitored and adjusted to help lower tissue hypoxia. Inserting a central venous catheter has many risk factors and is a big step to take. Early goal-directed therapy is a new concept being applied in order to prevent the negative outcomes of sepsis. Due to the delay in detecting

sepsis with lactate, this study is focusing on a coagulation biomarker to help decide if the patient's symptoms are severe enough for early goal directed therapy

2.3 Lactate as a Gold Standard

Clinical evaluation and laboratory testing are the primary sources of information for any patient with the potential of being diagnosed with severe sepsis or septic shock. These findings are specific to signs of infection, organ dysfunction and global tissue hypoxia. The laboratory data obtained are used as biomarkers. Any laboratory abnormalities that are within the criteria for sepsis can be used in aiding the correct diagnoses and treatments. An example of an initial test that is run is a complete blood cells count (CBC) that includes a standard chemistry panel of bicarbonate, liver enzymes, lactate, creatinine, white blood cells (WBC) and coagulation studies. CBCs are an extremely vital indicator of the present of bacteria and severity of infection. If the WBC count is high it usually indicates the presence of an infection. If lactate levels are high it indicates global tissue hypoxia. Creatinine levels indicate kidney function. The CBC is usually run regardless of the initial symptoms in order to see what is going on with the biochemistry within the body, and can at times explain the symptoms being seen. The downside to using a CBC as an indicator of a bacterial infection is its lack of specificity. Therefore the CBC alone cannot be used to make a final diagnosis.

Among the estimated 80 different biological markers for sepsis, lactate levels are currently the main test for global tissue hypoxia. In a majority of instances, elevated lactate levels in the emergency department due to an infection leads to a poor prognosis for the patient (Talan, 2008)

2.3.1 Lactate- How it Works Within The Body

The normal plasma lactate concentration is approximately 0.3-1.3 millimol/L. During normal lactate production glycolysis in the cytoplasm produces pyruvate, which is then converted into acetyl CoA during aerobic conditions. The acetyl CoA then enters the Krebs cycle which produces 36 ATP via the electron transport chain. Lactate is produced when anaerobic conditions are present. The pyruvate is converted into lactic acid by the enzyme lactate dehydrogenase. The lactic acid is then dissociated into lactate addition of 2 hydrogens, resulting in the regeneration of NAD from NADH. However, acidosis can occur if the oxidative pathway is impaired and a surplus of H+ is present. NADH also controls the conversion of pyruvate to lactate. Tissues that require a lot of ATP need the cells to go through aerobic metabolism, therefore the NADH levels need to be kept low. The liver removes approximately 70% of the lactate by metabolizing it and releasing it into the bloodstream (Bolton, 2007). This is the lactate produced during anaerobic metabolism (Phypers, 2006). This process is demonstrated in Figure 1 below.

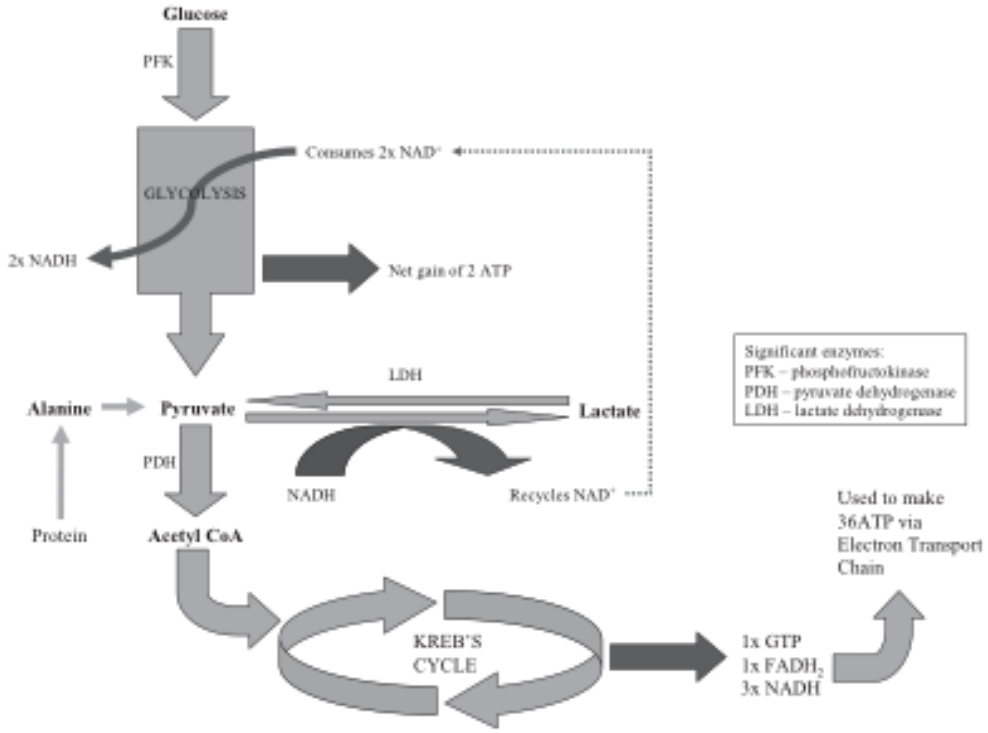


Figure 1: Glycolysis, Kreb's Cycle, and Oxidative Phosphorylation (Phypers)

2.2.2 Lactate and Sepsis

High levels of the lactate produced during anaerobic metabolism are used as the main indicator for tissue hypoxia. The anaerobic pathway involves metabolism of lactate back into pyruvate by well-oxygenated organs like the liver, heart kidneys and brain. In cases of sepsis or septic shock, when the organs become hypoxic, the production of lactate exceeds the rate at which an organ, such as the liver can metabolize it. Therefore the lactate builds up in the bloodstream, and basis of lactate measurement is from the blood (Bolton).

Another reason for high lactate levels in patients with sepsis could be due to Type A lactic acidosis. Type A lactic acidosis is when there is decreased oxygen delivery or increased oxygen demand. This belief has been challenged over the past

decade. If this were true and hypoxia were the primary reason for the increase in levels, stimulation with pyruvate dehydrogenase (PDH) by dichloroacetate should have been able to decrease the levels of pyruvate and lactate.

2.3 Coagulation

2.3.1 Coagulation and Inflammatory Cascades

The current treatments for sepsis using antibiotics and vasopressors are limited in decreasing the mortality rate. In order to establish a more appropriate course of treatment, it is important to study the pathophysiology and cellular mechanisms involved in the inflammatory response related to sepsis.

Endotoxin is a molecule derived from gram-negative bacteria's membrane. It is the initial stimulus that causes the cellular release of cytokines. It has the ability to activate macrophages and endothelial cells, and activate the release of proinflammatory mediators such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6), high-mobility group box-1 protein (HMGB-1), macrophage migratory inhibitory factor (MIF), platelet activating factor (PAF), nitric oxide (NO), and ecosonoids. Figure 2 below displays the interaction.

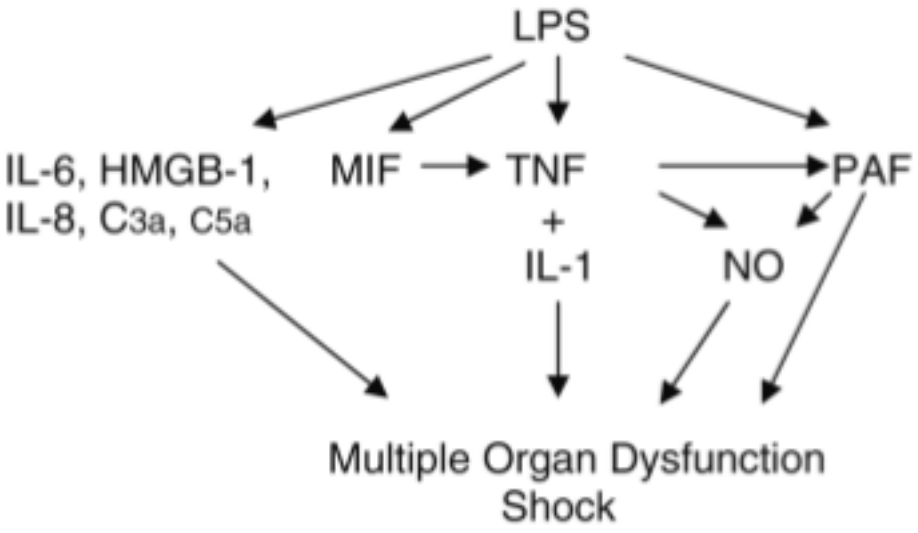


Figure 2: Activation and Interaction of Inflammatory Mediators in Sepsis (Jean-Baptiste,2007)

LPS binds to the surface molecule CD14 on macrophages. CD14 lacks an endoplasmic domain; therefore, signaling is done through a transmembrane protein known as a "toll-like receptor" (TLR). There are currently 10 TLR's that have been identified. Both the IL-1 receptor and TLR share the same endoplasmic domain and have similar apoptotic effects. TLR-4 recognizes gram-negative bacteria. Binding of the LPS/CD14 complex to TLR-4 activated nuclear factor kappa-beta through phosphorylation. Under normal conditions, NF-kb is in the cytoplasm and essentially inactive. The nuclear translocation of NF-kb promotes the production of cytokines via transcriptional regulation of target genes. The visual schematic of this can be seen in Figure 3 below.

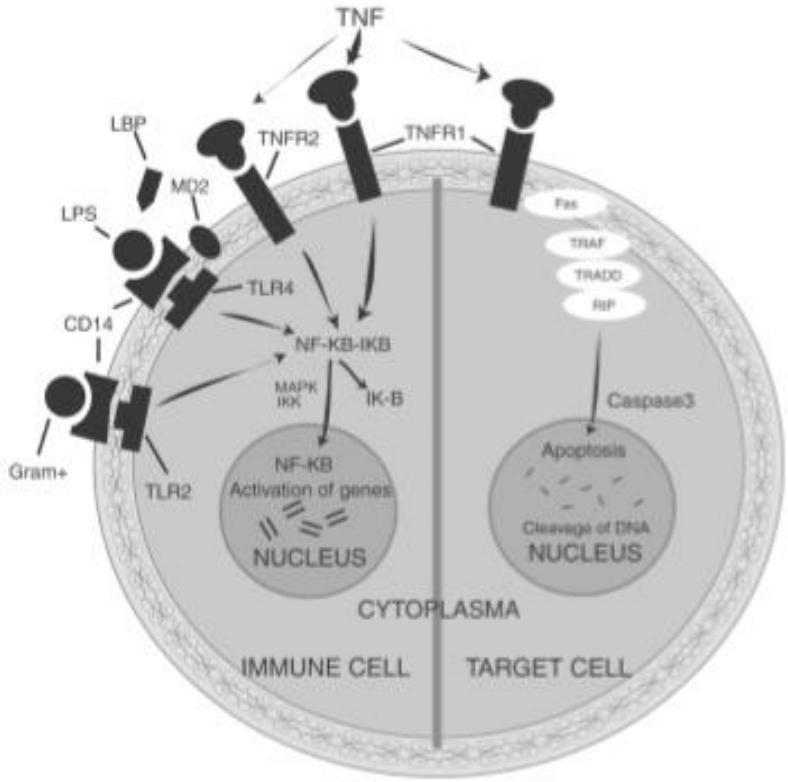


Figure 3: Cellular Responses to Infective Agents and TNF in the Immune and Target Cells (Jean-Baptiste).

2.3.2 Platelet Function in Coagulation

As part of the coagulation cascade, platelet function plays large role, specifically in relation to non-specific inflammatory defense during times of severe infection. A previous study was done to look at platelet function in septic multiple organ dysfunction syndrome (MODS). The study evaluated adhesive functions of platelets in MODS in patients in the ICU. It was found that platelets are activated in septic patients and significant degranulation was found only in MODS patients (Yilamaz, 2005).

Platelet closure time (PCT) was first evaluated as a potential biomarker in a study performed with dogs. The study looked at the importance of PCT in dogs with

endotoxemia (Bolton, 2007). The study found that short closure time has the potential to serve as an early diagnostic biomarker for endotoxemia and longer closure times could be used as a gauge of the severity of endotoxemia (Yilamaz, 2005).

2.4 Platelet Closure Time and Severe Sepsis at UMass Medical School

The hypothesis of this study is that platelet closure time will aid in identification of severe sepsis and septic shock in humans at an early stage and the degree of platelet closure time will identify the severity of the disease. A broad spectrum of patients will be enrolled and the severity of their illness will be determined by an APACHE II score (described in section 2.4.1 below). The platelet closure times will be analyzed along with their lactate levels to determine if there is any correlation.

2.4.1 Platelet Closure Time

To establish platelet closure time in a blood sample, a platelet function assay (PFA) must be performed. The test measures both platelet adhesion and aggregation. The PFA test is a rapid and accurate test of platelet function. The template bleeding time often gives poor and irreproducible results. The PFA test is performed with a laboratory analyzer that is known as the PFA-100. Small membranes that are coated with collagen and epinephrine or collagen and ADP are used. A blood sample is then passed through the membranes at rate to simulate the hemodynamics in the small capillaries. The platelets adhere to the membranes and form a small aperture in the center of each membrane. The time, in seconds, it takes

to close the aperture is known as “closure time” (CT). The results of this test and what a normal closure time should be are still trying to be determined.

2.4.2 Physiologic Scoring Systems

In order to compare the severity of illness of patients, this study uses the APACHE II system in order to score each patient’s severity of illness. The score directly correlates to a mortality rate prediction. Physiologic scores are assessed to compare illness severity immediately upon arrival to the Emergency Room, although this is not routinely done. It is crucial that these physiologic scores be obtained in the most proximal stage of the disease. Often by the time the patient is transferred to the ICU the scores may be higher or lower, due to the progression of the disease or the therapy administered. The initial physiologic scores that are evaluated in order to calculate the APACHE II score are: age, mean arterial pressure (MAP), serum potassium, serum sodium, hematocrit, WBC, serum creatinine, serum pH, bicarbonate levels, temperature, respiratory rate, and heart rate (Otero, 2006).

3.0 Methodology

3.1 Admission of Patients

The target enrollment number of patients in this study is 50. The first 3 patients were enrolled in 2009, these were the only patients enrolled before we started enrolling patients. The patients enrolled will range in the degree of illness. APACHE II scores are used to estimate mortality rate due to different signs established near the time of blood draw. Over the course of this phase of the study

from September 2010 through April 2011 (or whatever the real dates are), twelve patients were enrolled including the 3 in 2009. There were 15 blood samples drawn, due to the fact that three of the patients had two draws, peripheral and central.

3.1.2 Patient criteria

In order for a patient to be eligible for the Platelet Closure Time Study they must have had at least two SIRS symptoms and presence of infection. The SIRS symptoms can be observed in Figure 3 below.

1. Temperature	<36° C or >39°C
2. White Blood Cell Count	>12,000 or <4,000 or >10% bands
3. Heart Rate	>90 bpm*
4. Respiratory Rate	>24 bpm^ or $PaCO_2$ <32mm Hg
Sepsis = SIRS + infection	
Severe sepsis = SIRS + infection + end organ damage	
Septic shock = Severe sepsis + refractory hypotension (<90 mm Hg or 40% below baseline) * beats per minute ^ breaths per minute	

Figure 4: SIRS Criteria

These criteria allow for a broad spectrum of patients to be included. Some are enrolled with only SIRS symptoms while others already have severe sepsis or septic shock. The spectrum of different participants will give the study more merit and allow a more complete analysis of the relationships between sepsis and platelet

closure time. Also incorporated in the study are exclusion criteria; patients must be 18 year of age or older and pregnant patients and prisoners are not eligible.

3.1.3 Obtaining patient consent

After it was determined that a patient fit the criteria for the study, patient consent needed to be obtained prior to enrollment. There were two consent forms that needed to be signed by the participant and the primary investigator, Dr. Marie Mullen. The first form was to gain consent to analyze platelet closure time of previously drawn blood. The second form was to gain access to their medical records. Both of the consent forms needed to be signed before the patient was able to be enrolled in the study. Copies of the consent forms were given to the patient to keep.

The patient also had to be eligible to consent. If they were not coherent enough to consent themselves, a family member could consent on their behalf. This also applied to mentally ill patients.

3.2 Blood Analysis

Once the patient consented, the researchers checked with the nurse to determine if there were orders to draw blood. If so, an extra blue top tube of blood was requested to be drawn. The first blood sample received was usually from a peripheral draw within the first hour of the patient entering the Emergency Room. A second blue top tube was obtained if a central venous catheter was inserted. This typically occurred when a patient is diagnosed with severe sepsis or septic shock and early goal-directed therapy was needed.

3.2.1 Analyze samples with PFA-100 within 24 hours of blood collection to obtain CT values for EPI cartridge and ADP cartridge

The Platelet Function Analyzer (PFA-100) was used to analyze the blood collected in the blue top tube within 24 hours of the draw. First a blank is run, and then the ADP and EPI cartridges are loaded. Using a micropipette, 800 microliters of blood is loaded into each cartridge. The sample is then run through the machine in order to measure the platelet closure times.

3.3 Results Interpretation

As patients were enrolled into the study they each had a data collection sheet with their assigned research number on it. The PFA-100 platelet closure time values were noted along with age, sex, anticoagulants taken, and the time of the blood draw. The results of the following tests ordered by the physician were also documented: lactate, ScVO2, platelet count, Partial Thromboplastin Time (PTT), Prothrombin Time (PT)/ International Normalized Ratio (INR), creatinine, and blood urea nitrogen count (BUN). All of this information was also entered into an excel spreadsheet.

3.3.1 Information Obtained From Patient Records

From the patient's medical records the time in the Emergency Department was recorded along with the length of stay in the Intensive Care Unit, the length of stay in the hospital, the number of days on a ventilator, and the amount of time on vasopressors. This information allows the researchers to follow the course of treatment for each patient and how their illness progressed.

Each patient was given an APACHE II score in order to compare the degree of illness for each patient in the study. In order to calculate the score using an electronic calculator the following information had to be obtained from the patient's chart:

-Age	-Respiratory Rate
-Mean Arterial Pressure	-Heart Rate
-Temperature	-Serum Sodium
-Serum Potassium	-Serum pH
-Bicarbonate	-Serum Creatine
-Blood Gas (if applicable)	-Glascow Score

From the information entered an estimated mortality rate was calculated. This allows the study to compare the severity of illness for each patient. This additional information gathered was entered into a second Excel spreadsheet.

3.3.2 Statistical Analysis

There were a total of 12 cases and 15 blood draws. The patients were placed into two different groups based on their lactate levels. The first group had high lactate levels (4 or greater). The second group had normal or low lactate levels (lower than 4). The descriptives, such as: mean, standard deviation, minimum and maximum, variance, and range for each of the groups were calculated using SPSS. The Kolmogorov-Smirnov test of normality was performed to determine how to optimize the analysis by using parametric or non-parametric tests. Histograms show a graphical representation of both ADP and EPI values for both of the groups, high lactate and low lactate. Box-plots were also generated and show each groups variability. To analyze if the difference was due to chance or not, an independent T-Test was run. The null hypothesis was that there was no real difference between the

two groups' platelet closure times. The assumptions of the test were that there was normality and similar variances in the two groups. As stated before, a Kolmogorov-Smirnov Test was run to determine if the normality assumption could be made. Then the Levene's Test was run to determine if the similar variances assumption could be made.

3.3.2.1 Association of PCT and Lactate

A linear regression analysis was used to determine if there was an association between platelet closure times and lactate levels. The dependent (outcome) variable was platelet closure time, which was continuous data. The independent (predictor) variable was lactate levels, which was also continuous data. The assumptions were that the relationship between two variables should be linear, the dependent variable, Y, distributed normally, and the variability of Y as assessed by variance or standard deviation should be the same for each value of X proven. The linearity of the relationship was shown using a scatter plot. The normality of the dependent variable, platelet closure time, and the variance were determined using the Kolmogorov-Smirnov Test and the Levene's test, as described above. The null hypothesis was that there was no relationship between the two variables. The linear regression was run to determine if the null hypothesis could be rejected.

In order to determine if there were any confounding variables, gender, age, peripheral or central draw, platelet count, BUN and creatinine levels were noted for each patient in the two groups. These variables were compared with platelet closure time using linear regression analysis. The null hypothesizes for each was that there

was no relationship between platelet closure time and any of the additional variables. These results were all described in the results section.

4.0 Results and Analysis

Twelve patients were enrolled into the study with 15 blood draws. The 12 patients were divided into two groups based upon their lactate levels. Patients in group 1 (seen throughout the SPSS figures below as group .00) the less sick patients, had lactate levels below 4 millimol/L. The second group is comprised of sicker patients, with lactate levels of 4 millimol/L or higher. This is a very small sample size and therefore, difficult to interpret. The following tests are all not statistically significant most likely due to the fact of the sample size. The tests were run with this data set in order to show the statistical analysis that would be used when the study is complete.

The data collection Excel sheets with all the data collected for the study, are included in Appendix 1. It is difficult to determine whether or not there are any trends when looking at the data in this form. Before the data could be analyzed graphically the descriptives of the data needed to be determined, as seen in Table 2 below. This includes values such as: mean, confidence intervals, median, variance, minimum, maximum, and range. This was done for both types of platelet closure times, ADP and EPI, as well as for both groups, high and low lactate levels.

For the ADP platelet closure times there was not a large difference between the means of the two groups. Group 1 had an average of 142.3 seconds and Group 2 had an average of 150.5 seconds. There was a greater difference in the medians

between the two groups. With a small sample size the median can sometimes be a better representation than the mean. The EPI means were somewhat more varied but still not drastically different. The medians were also somewhat different but not drastic. This does not give a conclusive outlook on whether or not the sicker patients with higher lactate levels have longer platelet closure times.

Table 2: Platelet Closure Time Group Descriptives: the group indicated as 0.00 is the low lactate group, while the group indicated as 1.00 is the high lactate group.

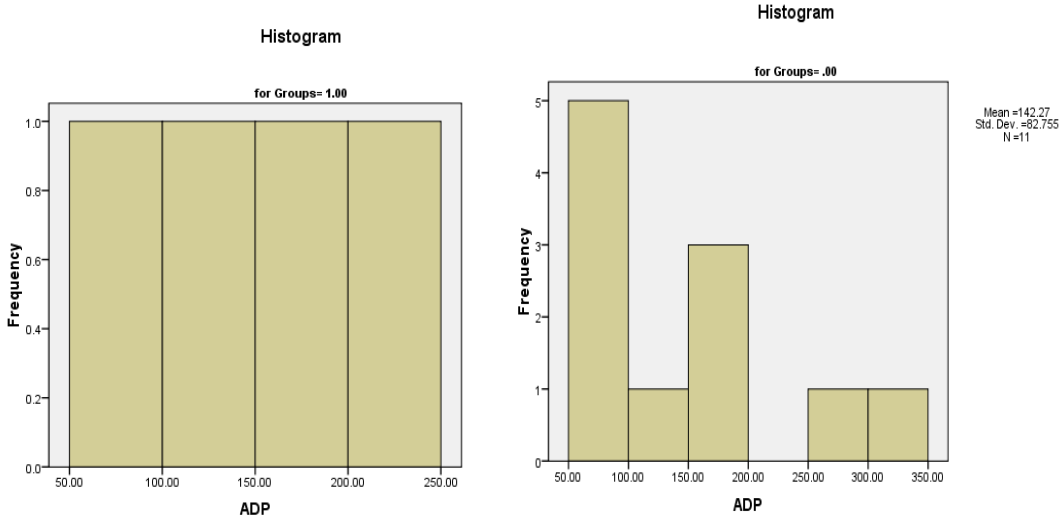
Descriptives

	Groups		Statistic	Std. Error
ADP	0.00	Mean	142.2727	24.95162
		Median	132	
		Variance	6848.418	
		Std. Deviation	82.75517	
		Minimum	65	
		Maximum	300	
		Range	235	
		Interquartile Range	108	
		Skewness	1.007	0.661
	1.00	Mean	150.5	39.20991
		Median	148.5	
		Variance	6149.667	
		Std. Deviation	78.41981	
		Minimum	65	
		Maximum	240	
		Range	175	
		Interquartile Range	151	
		Skewness	0.101	1.014
EPI	0.00	Mean	183.3636	25.60301
		Median	196	
		Variance	7210.655	
		Std. Deviation	84.91557	
		Minimum	68	
		Maximum	300	
		Range	232	
		Interquartile	162	

		Range		
		Skewness	-0.094	0.661
	1.00	Mean	227	15
		Median	226	
		Variance	900	
		Std. Deviation	30	
		Minimum	196	
		Maximum	260	
		Range	64	
		Interquartile Range	57	
		Skewness	0.104	1.014

4.1 Graphical Analysis

Once the values were determined, histograms for both ADP and EPI PCT's were generated in order to give a better graphical representation of the difference between the two groups.



A) Group 1 (low lactate)

B) Group 2 (High Lactate)

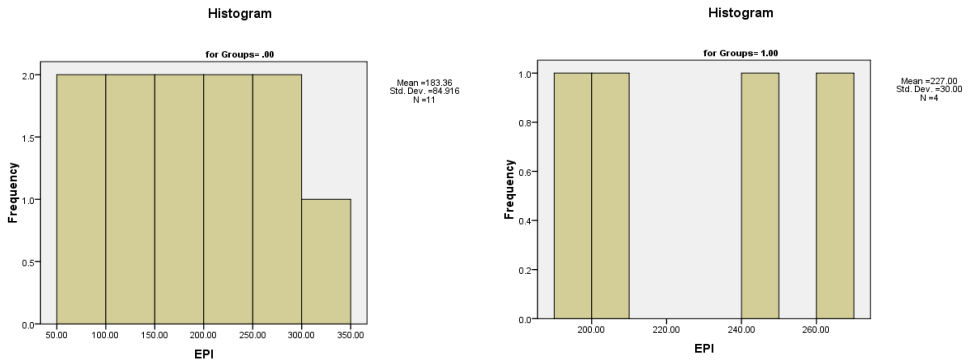
Figure 5: ADP Platelet Closure Time (seconds) for groups 0.00 (low lactate levels) (A) and 1.00 (high lactate levels) (B). This shows the distribution of PCT's within each group.

As can be seen in Figure 5, ADP platelet closure times across the patient samples ranged from a low of 65 seconds to a high of 300 seconds. In the low lactate group, none of the eleven patients had levels below 200 seconds and the remaining two had values above 250 seconds. However, in the high lactate group, 3 of the 4 had lactate levels below 200, representing about an equivalent fraction as in the low lactate group. The means for the two groups, 142 seconds and 150 seconds do not appear to be remarkably different and there is no discernable change in the pattern or distribution of values between the two groups.

By contrast, in the EPI data for platelet closure time, 54% of the low lactate group had PCTs below 200, compared to only 25% of those in the high lactate group. While these data are not statistically significant due to the small sample size and the uneven group sizes, the trend is in keeping with our hypothesis that platelet closure time will be higher in patients with higher lactate levels, suggestive of increased severity of sepsis.

In Figure 5A, the histogram for the first group's ADP platelet closure times is somewhat skewed toward the left. This was also seen above in Table 2 with the skewness values. Even though it seems skewed, the differences may not be statistically significant. A test of normality will tell whether or not it is truly skewed. When a sample size is extremely small the histogram appearance can be deceiving. Figure 5B does not give us too much information as far as skewness and this is due to the fact that group 2 only has four values, all of which are different. With such a

small sample size in a group, skewness is not immediately evident from a histogram. Skewness tells how much the data trend sways from normality. Positive skewness indicates the data is skewed to the right and negative skewness indicates that the skewed to the left. Normality indicates the data has a bell curve trend. This is important because normality is an assumption for many statistical tests.

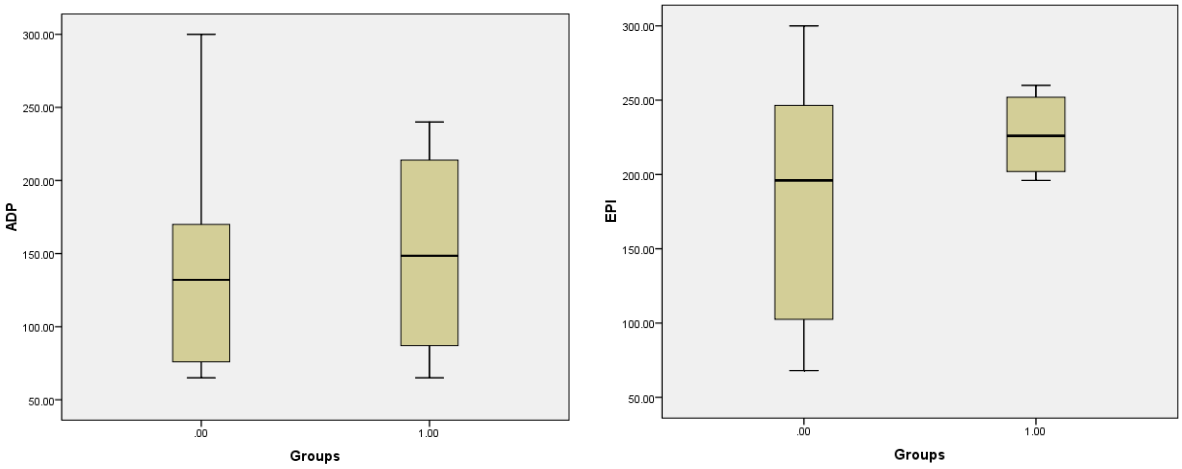


A) Group 1 (Low Lactate) B) Group 2 (High Lactate)

Figure 6: EPI Platelet Closure Time Histograms for Groups 1(A) and 2(B). This shows the distribution of PCT's within each group.

Figure 6A and 6B show histograms for the EPI platelet closure times. Neither of the graphs shows an obvious skewness or trend in the data. It does show a hopeful trend in the fact that half of the low lactate patients has levels <200 seconds while none of the high lactate people did. This is not significant at the time but seeing a trend such as this early on can be potentially promising. This is due to the lack of data in the small sample size. It appears in this case that the EPI is not likely to be as sensitive as the ADP due to the fact that the trends below are more general than seen above. This could possibly be due to chance and thrown off by the small sample size. A definite conclusion would be able to be drawn about the difference

between the ADP and EPI platelet closure times if the sample size where significantly larger than the current size of 15. ADP and EPI are different platelet mediators and therefore, could potentially have somewhat different affects on the platelets and PCT.



A) ADP Platelet Closure Times B) EPI Platelet Closure Times

Figure 7: Box and Whisker Plots for both ADP and EPI Platelet Closure Times. This graphical representation is used in order to show variability. Figure A, shows ADP's PCT's for both group 0.00 (low lactate) to the left and group 1.00 (high lactate) to the right. Figure B, shows EPI's PCT's for both groups as well.

Figure 7 shows box and whisker plots for both ADP and EPI platelet closure times. Box and Whisker Plots are effective in order to show variability and outliers within the two groups. Figure 7A shows that there is only a slight difference between the two groups. Group 1 has a significant outlier at 300 seconds where group 2's values have less variability. If the outlier were removes the mean for the low lactate group would be lower and the trend would be consistent with the hypothesis. This once again shows that the current trends, even with the small

group size, are favoring in the right direction. It would have been ideal to see group 1 with a lower median around 80 seconds and group two with a higher median around 250 seconds. The outcome we predicted is not seen here with our present data. The high lactate group (1.00) is, however, somewhat higher. With more patients enrolled in the study in the future it is possible that these two groups will differentiate from each other more as predicted in our initial hypothesis. Figure 7B below shows the low lactate group (0.00) does have a lower median than the second group, as established in Table 2. However, there is some of variability in group 0.00. With a greater sample size a better understanding would be able to be established. The graphic presentation gives a good outlook on potential trends. The statistical data does not tell us that without the outlier the data would look very similar to what was predicted with our hypothesis. According to the graphical representation as more patients are enrolled the statistical data will most likely improve as well.

4.2 T-Test

An independent t-test compares the mean scores of two groups on a given variable. It is used to determine if the difference between the means is due to chance or if there is a statistically significant difference. A statistically significant difference in this case would mean that there is a difference in platelet closure times between sicker patients with higher lactate levels and less sick patients with lower platelet closure times.

4.2.1 T- Test Assumptions

A Kolmogorov-Smirnov test of normality was used to see if the data is normally distributed or skewed. An idea of normality can be obtained from the histograms usually but due to the very small sample size it was nearly impossible to determine whether or not the data was truly normal by looking at the graphs. The null hypothesis is that the data distribution is normal. For both ADP and EPI both groups there was not enough information to reject the null hypothesis because the p-values shown in the Sig. column were greater than 0.05, therefore the null hypothesis was accepted. This means that the test is 95% confident that the data does follow a normal trend. In both cases group 1 did not produce a p-value. This is because the sample size is only 4 in this group and it is not large enough to make a true determination. This data indicated that the following statistical tests needed to be parametric tests. Non-parametric tests are typically used for small data sets; however, the assumptions for parametric tests were met, which indicated that parametric tests were needed.

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Groups	Statistic	df	Sig.	Statistic	df	Sig.
ADP	.00	.225	11	.125	.846	11	.037
	1.00	.202	4	.	.965	4	.809
EPI	.00	.189	11	.200*	.917	11	.294
	1.00	.237	4	.	.925	4	.565

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Figure 8: Kolmogorov-Smirnov Normality Test for both ADP and EPI platelet Closure Times. The Sig column indicates that for both groups the null hypothesis of normality is accepted.

Figure 9 is a two-sample Kolmogorov-Smirnov Test. Instead of splitting ADP and EPI into groups 1 and 2 it tested if ADP or EPI as a whole had normal data. The null hypothesis was that the data follows a normal trend. In both cases there was not enough evidence to reject the null hypothesis because the p-values were greater than 0.05. This confirms the fact that parametric tests need to be used when testing this data set. The first assumption of a t-test is that the data follows a normal trend. This was verified by figures 8 and 9.

The second assumption of a t-test is that the two groups being tested have approximately equal variance for the dependent variable, in this case platelet closure time. Figure 11 shows the Levene's Test of equality of variances. The null hypothesis is that the variances are equal. For ADP the p-value is above 0.05 and the null hypothesis can be accepted. For EPI the p-value is less than 0.05 and the null hypothesis is rejected. The assumption is only valid for ADP platelet closure time values. The discrepancy between the two adhesion factors is probably not significant and only seen due to the small sample size at this time.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
ADP	Equal variances assumed	.001	.970	-.172	13
	Equal variances not assumed			-.177	5.644
EPI	Equal variances assumed	6.118	.028	-.985	13
	Equal variances not assumed			-1.471	12.955

Figure 9: Levene's Test for Equality of Variances. The Sig Column indicates that equal variances are assumed for ADP, but not for EPI. EPI cannot assume equal variances as a result from this test.

4.2.2 Independent T-Test Results

The null hypothesis is that the two groups are not significantly different. For ADP equal variances can be assumed and the p-value seen in figure 10 is higher than 0.05, so the null hypothesis is accepted. For EPI equal variances cannot be assumed, the p-value is still higher than 0.05 so the null hypothesis is accepted again. The conclusion from this test is that at this time there is no difference in platelet closure time between patients with high lactate levels and patients with low lactate levels. With a higher sample number and more platelet closure times a difference between the group means could possibly be established.

Independent Samples Test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
ADP	Equal variances assumed	.866	-8.22727	47.74637
	Equal variances not assumed	.866	-8.22727	46.47580
EPI	Equal variances assumed	.343	-43.63636	44.29120
	Equal variances not assumed	.165	-43.63636	29.67346

Figure 10: Parametric Independent T-Test. This test established that at this time there is no difference in PCT's between sicker patients with high lactate levels and less sick patients with low lactate levels.

4.3 Linear Regression

A linear regression was done to see if there is an association between lactate levels and platelet closure time. The hypothesis of the study is based on this assumption. The predictor values were the groups because they were separated based on lactate levels. The higher lactate levels are in Group 2, the lower in Group 1. Figure 11 shows the regression summary. The test is not statistically valid because the R squared value is very close to 0. If there is statistical significance the R squared value should be very close to 1. An assumption of the linear regression is that the data are nearly linear. If it were linear the following ANOVA's in Figure 12 would be able to truly determine if there was an association. With more test subjects a linear relationship with an R squared value may be able to be established.

A) ADP Linear Regression Summary

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.030[a]	.001	-.076	81.83206

a. Predictors: (Constant), Lactate

b. Dependent Variable: ADP

B) EPI Linear Regression Summary

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.480[a]	.230	.171	69.00473

a. Predictors: (Constant), Lactate

b. Dependent Variable: EPI

Figure 11: ADP (A) and EPI (B) Linear Regression Summaries. The conclusion from this test is that the data is not linear for either set of PCT's.

Figure 12 is of an ANOVA. This test is not valid due to the fact that the R squared values were not close to 1 in Figure 11. The null hypothesis is that there is no association between platelet closure time (ADP) and lactate. The p-value is extremely high and therefore, the null hypothesis is accepted. Once again, this result is possibly due to the small sample size.

A) ADP ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	77.410	1	77.410	.012	.916[a]
	Residual	87054.324	13	6696.486		
	Total	87131.733	14			

a. Predictors: (Constant), Lactate

b. Dependent Variable: ADP

B) EPI ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	18490.519	1	18490.519	3.883	.070[a]
	Residual	61901.481	13	4761.652		
	Total	80392.000	14			

a. Predictors: (Constant), Lactate

b. Dependent Variable: EPI

Figure 12: ADP (A) and EPI (B) ANOVA's. These ANOVA's prove that at this time there is no association between lactate levels and PCT's.

4.4 PCT and Mortality Analysis

A linear regression was performed in order to determine if there is an association between both ADP and EPI Platelet Closure Times and mortality. In this case the mortality rate for each patient was calculated by determining his or her APACHE II score (This process is discussed in the Introduction). Neither of the R

squared values is close to zero. This indicates that the data are not linear and the following ANOVA's in Figure 14 are not statistically relevant. This is however, the statistical test that would be used in order to determine if there is an association when more patients have been enrolled.

A) ADP Platelet Closure Times Vs. APACHE II Score (Mortality)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.126[a]	.016	-.060	81.21976

a. Predictors: (Constant), APACHEII

B) EPI Platelet Closure Times Vs. APACHE II Score (Mortality)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.208[a]	.043	-.030	76.91682

a. Predictors: (Constant), APACHEII

Figure 13: Linear Regression of Platelet Closure Time Vs. Mortality Rate (APACHE II Score). In both A and B the R squared value is close to zero indicating that the data is not linear.

Figure 14 below is of an ANOVA. This test is used when the linear regression shows the data is linear. This is the actual test that shows if there is an association. The null hypothesis is that there is no association between the two variables (PCT and mortality). In both A and B the p-values located in the Sig. column are over 0.05. This means that there is not enough evidence to reject the null hypothesis and therefore, it is accepted. With more patients enrolled this result could possibly change.

A) ADP ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1375.296	1	1375.296	.208	.655[a]
	Residual	85756.437	13	6596.649		
	Total	87131.733	14			

a. Predictors: (Constant), APACHEII

b. Dependent Variable: ADP

B) EPI ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3481.427	1	3481.427	.588	.457[a]
	Residual	76910.573	13	5916.198		
	Total	80392.000	14			

a. Predictors: (Constant), APACHEII

b. Dependent Variable: EPI

Figure 14: ANOVA between PCT and Mortality. The null hypothesis is accepted due to the high p-values in both A and B.

4.5 Confounding Variables Analyses

Different variables that are present in a study can at times have an effect on the study. Some of these variables can be gender, age, peripheral or central blood draw, time of blood draw, whether a doctor or nurse draws the blood. In any study you need to determine which variables could possibly have an effect on the study. Some will be more prominent than others. In this case, ANOVA's were performed between PCT and gender and PCT and age. This will tell if there is an association between the variables. If there is, then gender (or age) is a confounding factor and influencing the results of the study. If a variable is determined to be a confounding

factor it needs to be addressed in the study protocol and how the study is going to compensate for it.

4.5.1 Age

The Liner Regression in Figure 15 below indicates that the data is not linear. This does affect the ANOVA as stated above in Figure 13. With a larger sample size this can become more linear in trend.

A) ADP Linear Regression

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.103[a]	.011	-.066	81.43461

a. Predictors: (Constant), Age

B) EPI Linear Regression

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.265[a]	.070	-.001	75.83154

a. Predictors: (Constant), Age

Figure 15: Linear Regression for PCT verses Age. The R squared value indicated that the data is not linear.

In Figure 16 below the ANOVA's with both ADP and EPI platelet closure times indicates that there is no association between age and PCT. The null hypothesis is that there is not association. This is accepted because the p-values are higher than 0.05. Age is not a confounding factor at this time.

A) ADP ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	920.993	1	920.993	.139	.715[a]
	Residual	86210.740	13	6631.595		
	Total	87131.733	14			

a. Predictors: (Constant), Age

b. Dependent Variable: ADP

B) EPI ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5636.503	1	5636.503	.980	.340[a]
	Residual	74755.497	13	5750.423		
	Total	80392.000	14			

a. Predictors: (Constant), Age

b. Dependent Variable: EPI

Figure 16: ANOVA between PCT and Age. Age is not a confounding factor because the null hypotheisis is accepted.

4.5.2 Gender

The second possible confounding factor tested was gender. The linear regression for both ADP and EPI show that the data is not linear with the current sample size. This is show by the R squared values close to 0 in Figure 17.

A) ADP Linear Regression

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.401[a]	.161	.096	74.99585

a. Predictors: (Constant), Gender

B) EPI Linear Regression

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.451[a]	.203	.142	70.19588

a. Predictors: (Constant), Gender

Figure 17: Linear Regression between PCT and Gender. The data is not linear.

Gender is not a confounding factor at this time. The null hypothesis for the ANOVA's in Figure 18 below is that there is no association between PCT and gender. This hypothesis is accepted because the p-values are greater than 0.05.

A) ADP ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	14014.817	1	14014.817	2.492	.138[a]
	Residual	73116.917	13	5624.378		
	Total	87131.733	14			

a. Predictors: (Constant), Gender

b. Dependent Variable: ADP

B) EPI ANOVA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	16335.000	1	16335.000	3.315	.092[a]
	Residual	64057.000	13	4927.462		
	Total	80392.000	14			

a. Predictors: (Constant), Gender

b. Dependent Variable: EPI

Figure 18: ANOVA between PCT and Gender. There is no association therefore; gender is not a confounding factor at this time.

5.0 Conclusion

The previously stated hypothesis was that platelet closure time will aid in early detection of sepsis. At this time, with the number of patients enrolled in the study it is not possible to determine if the hypothesis is true. Currently, the statistics show that platelet closure time does not aid in early detection of sepsis. There is presently no association between the known biomarker, lactate, and platelet closure time. The first goal to validate the correlation between platelet closure time (PCT) and sepsis was to analyze the PCT in 50 patients that fit the study criteria. After a period of six months only 12 patients were enrolled into the study. There is still hope that when 50 patients have been enrolled the correlation between PCT and sepsis will be able to be validated with statistical analysis.

The subsequent goal is to establish a correlation between lactate as biomarker and PCT. As the results at this time indicate, the correlation between lactate and PCT is nonexistent. With more time and patients there will possibly be a change in the outcome of the related statistical analyses.

Even though the results to date are inconclusive this study can still produce results that will influence how sepsis is diagnosed. Due to the complexity of sepsis, early detection at this point is a challenge. If a larger sample size establishes that PCT does aid in early detection of sepsis by validating an association between PCT and lactate levels, the result of an early detection biomarker could lower sepsis mortality rates and potentially allow for more effective and cost-friendly treatments. On the other hand, if the larger sample size proves that there is no association between PCT and early detection of sepsis, the researchers can predict that the early detection biomarker is located further down the inflammatory cascade after the point where the coagulation cascade intersects.

At this time the statistical analyses are not significant due to the small sample size. However, the graphical representations are very promising. They show that the current trend is what the hypothesis predicted. If the trends continue in this manner as more patients are enrolled the outcome of this study is quite promising. It is difficult to enroll a lot of patients due to the exclusion criteria and other confounding factors that may affect the data, such as underlying disease or autoimmune disorders. Once the confounding factors are established outliers, such as the one seen in the box and whisker plots may be eliminated in order to gain a more accurate representation of the data. The study is still at an early stage but there are some hopeful indications for the future of the study.

6.0 Appendix

Table 3: Data Collection Sheet 1

ADP	EPI	Groups	APACHE II	Age	Gender
184	196	0	11	73	0
300	230	0	9	53	0
76	101	0	2	53	1
65	78	0	2	49	1
69	104	0	16	83	0
81	68	0	16	59	0
132	284	0	10	72	0
275	300	0	16	83	0
151	166	0	13	42	0
156	263	0	17	53	0
76	227	0	7	74	0
65	196	1	11	73	0
240	244	1	3	44	0
109	208	1	10	69	1
188	260	1	13	72	0

Table 4: Data Collection Sheet 2

Research Number	Time of Draw	Peripheral or Central	ADP(sec)	EPI(sec)	Lactate	ScVO2	Platelets	PTT	PT/INR	BUN/Cret
1001	1430	central	184	-	0.75/4.0			-	-	0/1.5
1002	1236	peripheral	>300	227	3.7	-	104	29.5	19.2/1.9	14/.66
1003	0200/0700	central	81/86	68/65	2.49	68%	243	-	38.8/3.4	43/8.5
1004	1320	peripheral	>300	terminated due to flow obstruction	0.9	-	97	37.4	10/.9	29/.77
1005	1100	peripheral	156	263	3.4	-	116	27.3	10.1/1.0	25/4.97
1006	1545/1818	peripheral/central	188/132	NA/284	5.7/2.57	NA/63%	142/120	28.7	13/1.2	17/1.19
1007	1425	peripheral	109/113	>208/>174	4.3	_	189	38.5	62/6.0	24/99
1008	1405	peripheral	>240(insufficient)	244	-	-	67	-	-	14/1.08
1009	1300/1600	central/central	>275/69	>300/104	3.0/1.51	67%/67%	319/297	38.7/NA	14/1.4/NA	92/3.61
1010	1351	peripheral	>151	>166	-	-	27	31.5	14.8/1.4	13/1.66
1011	1705	peripheral	76	101	1.1	_	268	25.3	10.7/1.0	14/0.95
1012	1619	peripheral	65	78	1.5	_	344	33.1	11/1.0	8/1.14

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