WHITE BLOOD CELL COUNT TRAJECTORY AND MORTALITY IN SEPTIC SHOCK: A

RETROSPECTIVE COHORT STUDY

by

Emily Rimmer

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ABSTRACT

Background: Septic shock is among the most common causes of admission to medical intensive care units (ICU) and is associated with mortality of 20-40%. The white blood cell count (WBC) at time of admission correlates with prognosis in septic shock but it is not known if the change in WBC over time (i.e. the WBC trajectory) impacts survival. Identifying unique groups of patients according to their WBC trajectories may provide valuable clinical prognostic information including response to treatments and level of care decisions.

Hypothesis: We hypothesized that the trajectory of the WBC count in septic shock can identify distinct clinical groups and be an independent predictor of 30-day mortality.

Objectives: 1) To identify groups of patients with different WBC trajectories using group-based trajectory analysis; 2) To evaluate patient and illness factors associated with different WBC count trajectories; and 3) To estimate the association of WBC trajectory with mortality in septic shock.

Methods: We completed a retrospective cohort study of adult patients with septic shock admitted to an ICU in Winnipeg, Canada between 2006-2014. We used group-based trajectory analysis to analyze the trend of WBC over the first 7 days of ICU admission to group patients according to statistically similar trajectories. Group-based trajectory analysis is a statistical method that can be used to describe the pattern of a variable over time. Rather than pre-specifying groups within a population, or using methods to measure an average trajectory for the entire population, group-based trajectory analysis allows for different groups with different trajectories to emerge. We used the Bayesian Information Criterion (BIC) and clinical validity characteristics to select the optimal trajectory model. We developed a multinomial logistic regression model to evaluate the association of patient and illness factors with WBC trajectories. We constructed a multivariable Cox proportional hazard models adjusted for age, Acute Physiology and Chronic Health Evaluation (APACHE) II score, comorbidities, infection type and antibiotics to evaluate the association of WBC trajectory on 30-day mortality.

Results: Our final study cohort comprised 917 patients with septic shock. The favored model identified 7 distinct trajectories of WBC (Figure 1). We found that baseline platelet count and sex were associated with WBC trajectory. The 30-day mortality of the entire cohort was 26.3%, and ranged from 23.1% in group 4 to 63% in group 5 (rising WBC trajectory). In a multivariable Cox proportional hazard model, group 5 was independently associated with an increase hazard of death (Hazard Ratio 3.41 (95% confidence interval 1.86 to 6.26), p<0.01).

Conclusions: We found seven unique and clinically relevant groups of patients with septic shock using trajectory analysis of the WBC count. Routine baseline characteristics are poor predictors of trajectory group assignment. The rising WBC trajectory is associated with an increased risk of death in septic shock. Further studies are required to fully describe the clinical characteristics and prognosis associated with distinct WBC trajectories and whether this information can inform level of care decisions and anticipated response to treatments.
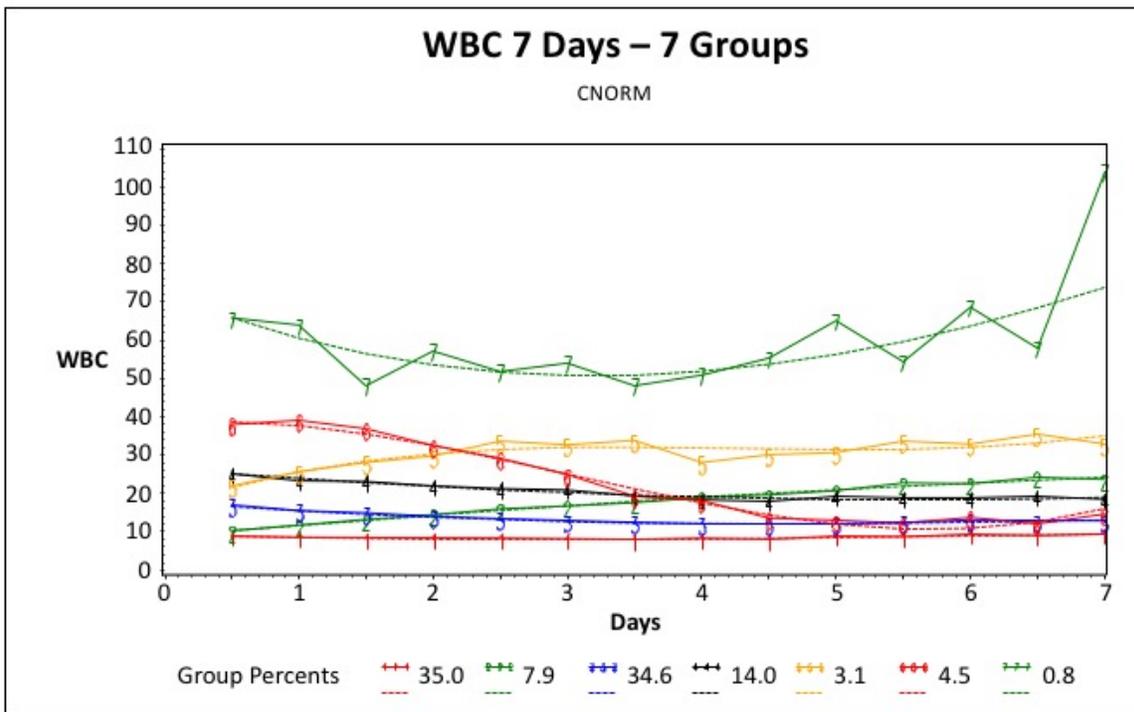
Figure 1: Trajectory of WBC in septic shock.

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ACCP – American College of Chest Physicians
AIDS – acquired immunodeficiency syndrome
ANOVA – analysis of variance
APACHE – acute physiology and chronic health evaluation
AvePP – Average Posterior Probability
BIC – Bayesian Information Criterion
CARS – compensatory anti-inflammatory response syndrome
CATSS – Cooperative Antimicrobial Therapy of Septic Shock
CBC – complete blood count
CI – confidence interval
CNORM – censored normal
CNS – central nervous system
COPD – chronic obstructive pulmonary disease HIV – human immunodeficiency virus
HR – hazard ratio ICU – intensive care unit
INR – international normalized ratio
IQR – interquartile range
NYHA – New York heart association
OCC – odds of correct classification
OR – odds ratio
RR – relative risk
SAS – Statistical Analysis Software
SCCM – Society for Critical Care Medicine
SD – standard deviation
SIRS – systemic inflammatory response syndrome
SOFA – sequential organ failure assessment
SSS – sepsis severity score
US – United States
WBC – white blood cell

CHAPTER 1 - INTRODUCTION

1.1 Introduction

Severe sepsis and septic shock represent an important burden on the health care system worldwide. The incidence of sepsis is approximately 3 per 1000 per year and the mortality of septic shock is 20% - 40%(1, 2). Septic shock affects people of all ages and ethnicities and affects individuals living in the community as well as those in acute or chronic medical facilities. The annual direct healthcare costs associated with severe sepsis or septic shock have been estimated to be $17 billion in the United States(3).

The concept and practice of personalized or precision medicine is transforming care in several health specialties and is relevant to the care of patients with septic shock. With a goal of delivering the *right* care to the *right* patient at the *right* time, precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle(4). One critical factor to this disease and treatment paradigm is the ability to recognize a patient's clinical trajectory so that appropriate treatment strategies can be considered – strategies that optimize treatment efficacy and risk based on the timing and severity of an illness in the context of predicted clinical outcomes and patient values and preferences. In the setting of septic shock, trajectory analysis may be an important tool to characterize these risks by identifying unique phenotypic clusters. As a proof of concept, this thesis aims to identify groups of patients with different white blood cell (WBC) count trajectories, identify patient and illness factors associated with different trajectories, and evaluate

the association of WBC count trajectory with mortality in septic shock. The unique groups of patients identified by their WBC count trajectories may provide valuable clinical prognostic information including response to treatments and level of care decisions. The unique groups identified will also be an important step to further research designed to evaluate disease biology and host genotypes and other factors that may impact clinical outcome and response to treatment.

1.2 The Epidemiology of Severe Sepsis and Septic Shock

The burden of illness of severe sepsis and septic shock is substantial. Using the original criteria of sepsis, severe sepsis and septic shock(5), in the United States 750,000 cases of severe sepsis or septic shock are estimated to be diagnosed annually and the incidence is rising(6). The incidence of sepsis rose by 8.7% annually between the years of 1979 and 2000(2). Similar trends are observed in Canada, where there is an estimated 30,000 cases annually, and hospitalization rates of severe sepsis over a 4 year period increased by 17.8%(7). Extrapolating from US data there are an estimated 20 million cases of sepsis treated worldwide per year and 10 million cases of severe sepsis based on a global population of 7.2 billion people(6, 8). Severe sepsis and septic shock represents approximately 10% of admissions to intensive care units (ICU) in the United States (6) and are among the leading causes of ICU mortality worldwide(2, 6, 9). Data on the epidemiology of sepsis is derived primarily from high-income countries and little is known about the incidence of sepsis in lower-income countries(8). Population-level incidence rates are available for the United States, Germany, Australia, Taiwan, Norway, Spain and Sweden which account for 7.2% of the global population. It is clear that little is known about the incidence of sepsis for more than 90% of the global population. The reported aggregate global incidence combining data from 2003-2015 is estimated to be 437 (95% confidence interval (CI) 334 – 571)

sepsis cases per 100,00 person-years(8). **Figure 1** shows the geographic distribution of known

population-level incidence data for hospital-treated sepsis and severe sepsis.

Figure 1. World map of included studies on sepsis and severe sepsis

The included studies present population-level incidence rates on hospital-treated sepsis and

severe sepsis (United States, Germany, Australia, Taiwan, Norway, Spain, and Sweden). Shaded

areas indicate countries in which the incidence of sepsis or severe sepsis is known. It is clear

that little is known about the incidence of sepsis is most areas of the globe. Reprinted from Am J

Respir Crit Care Med, 2016 with permission.

The incidence of severe sepsis in the United States is higher in infants and elderly patients

compared to other age categories, higher in men than women (relative risk (RR) 1.28 (95%

confidence interval (CI) 1.24 to 1.32), higher in blacks than whites (RR 1.89 (95% CI 1.80 to

1.98) and lower in Hispanics than whites (2, 6, 10). Contributing to the rising incidence of septic

shock is the increased presence of immunosuppression, chronic comorbid conditions and an aging population. In Canada, 60% of hospitalizations due to sepsis occur in patients over the age of 60 years with a median age of sepsis of 66 years(7).

Patients with sepsis have longer ICU lengths of stay compared to those who are admitted due to other reasons. The median length of stay in an ICU for patient with sepsis in Canada is 6.4 days which is about 4 days longer than for other admission diagnoses(7). The median ICU length of stay for severe sepsis in Canada is 9.5 days. These factors contribute to the high costs associated with the treatment of sepsis.

1.3 Definitions of Sepsis, Severe Sepsis and Septic shock

Sepsis is a term developed to characterize the signs and systemic symptoms that develop in response to infection(5). These signs and symptoms are variable and include non-specific findings such as fever, and changes in vital signs (blood pressure, respiratory rate, heart rate). Due of the non-specific nature of the symptoms as well as the variability in terminology, in 1991 the American College of Chest Physicians (ACCP) and the Society for Critical Care Medicine (SCCM) held a conference to develop a consensus definition of sepsis and related conditions. These consensus criteria were developed to assist with bedside diagnosis and early implementation of therapy as well as to standardize research protocols and nomenclature(5). During this meeting, the term systemic inflammatory response syndrome (SIRS) was coined (*Section 1.4 The Pathophysiology of Sepsis*). The group recognized that SIRS could develop

secondary to infection but also in response to other highly inflammatory states such as trauma, pancreatitis, and burns. The SIRS response is manifested by 2 or more of the following criteria:

(1) temperature greater than 38°c or less than 36°c;

(2) heart rate >90 beats per minute;

(3) respiratory rate >20 breaths per minute or paCO2 <32 mmHg;

(4) white blood cell count >12 x 10e9/L, <4 x 10e9/L, or >10% immature forms(5).

Sepsis was initially defined as systemic inflammatory response (SIRS) due to an underlying infection(5). Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion abnormality (lactic acidosis, oliguria, or altered mental status), or sepsis-induced hypotension (systolic blood pressure <90mmHg or >40mmHg reduction from baseline in the absence of other causes). Septic shock was defined as sepsis-induced hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities(5). The relationship of these entities is shown in **Figure 2**.

BACTEREMIA

INFECTION

FUNGEMIA

PARASITEMIA

VIREMIA

OTHER

SEPSIS

SIRS

OTHER

TRAUMA

BURNS

PANCREATITIS

BLOOD BORNE INFECTION
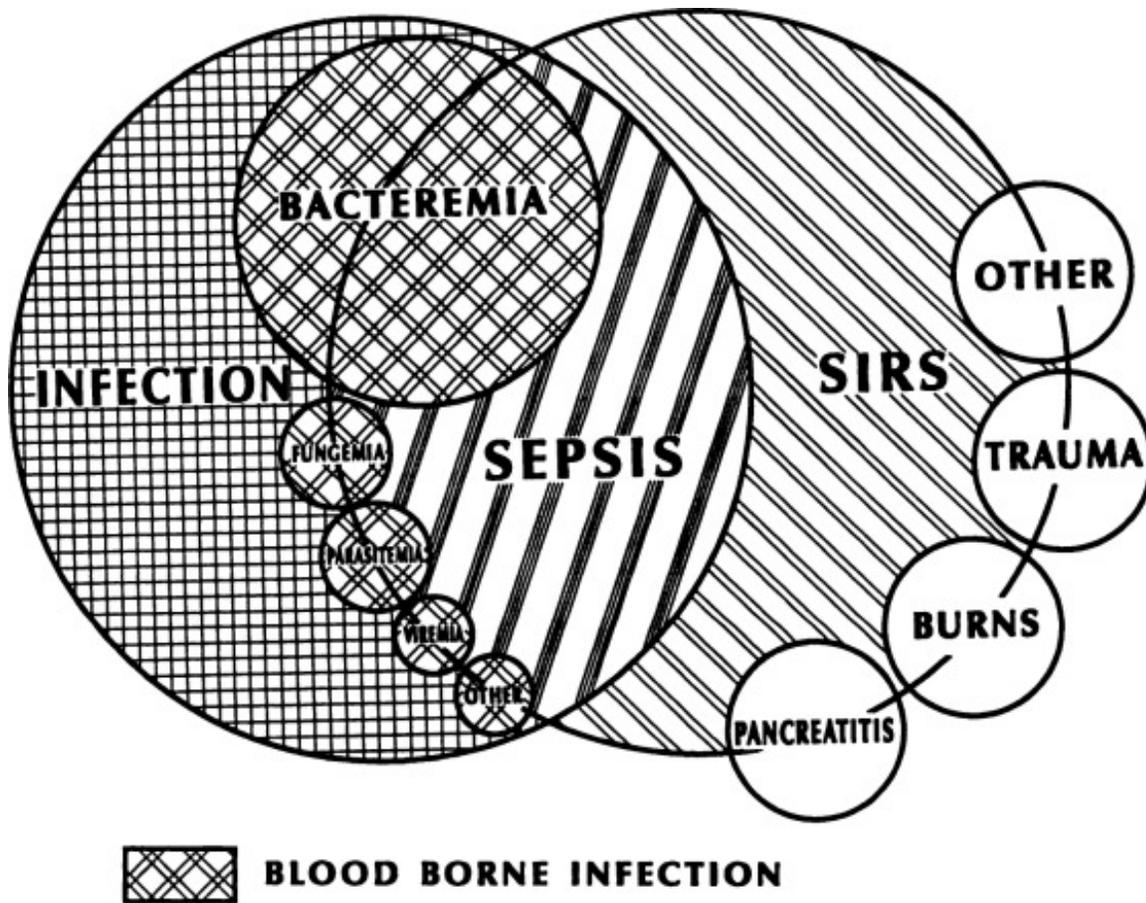
Figure 2: The interrelationship between SIRS, sepsis and infection.

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These definitions became widely adopted both in clinical practice as well as for inclusion criteria in clinical trials and research databases. In 2001, an expert panel of leaders from 5 international societies met to review the 1991 ACCP/SCCM sepsis criteria. Apart from expanding the symptoms and signs of sepsis beyond the SIRS criteria (**Table 1**), the definitions of sepsis, severe sepsis and septic shock remained unchanged(11).

Table 1: Diagnostic Criteria for Sepsis

Sepsis requires documented or suspected infection and at least one of the following:

General variables	Temperature >38.3^{o}c or <36°c
	Elevated heart rate (>90 beats per min)
	Increased respiratory rate
	Altered mental status
	Edema or positive fluid balance
	Hyperglycemia
Inflammatory variables	WBC >12 x 10e9/L or <4 x 10e9/L
	Normal WBC with >10% immature forms
	Elevated C-reactive protein
	Elevated procalcitonin
Hemodynamic variables	Hypotension (SBP <90mmHg, MAP <70mmHg, decrease in SBP >40mmHg
	Elevated mixed venous oxygen saturation
	Cardiac index >3.5
Organ-dysfunction variables	Hypoxemia (P:F ratio <300)
	Acute oliguria
	Creatinine increase by >44umol/L
	Coagulation abnormalities (INR >1.5 or PTT >60 seconds)
	Paralytic ileus
	Thrombocytopenia (<100 x 10e9/L)
	Hyperbilirubinemia
Tissue-perfusion variables	Hyperlactatemia (lactate >1mmol/L)
	Decreased capillary refill or mottling

WBC=white blood cell; SBP=systolic blood pressure; MAP=mean arterial pressure; P:F ratio= PaO$_2$:FiO$_2$ ratio; INR=international normalized ratio; PTT=partial thromboplastin time

The 1991 definitions for sepsis, severe sepsis and septic shock were considered to be the standard for more than two decades. In light of what was purported to be a more informed understanding in the pathophysiology of sepsis, a third consensus conference took place in 2014. Based on data that suggested that the SIRS criteria are neither sufficiently sensitive nor specific in the diagnosis of sepsis(12), the group unanimously agreed to remove the SIRS criteria from the definition of sepsis. In 2016, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) were published (13), and sepsis is now defined as "life-threatening organ dysfunction due to a dysregulated host response to infection" (13-15). Because sepsis is recognized as an entity separate from infection with an inflammatory response and requires evidence of organ dysfunction, the term severe sepsis was determined to be redundant. Septic shock is defined by sepsis and documented hypotension refractory to intravenous fluids or associated with hyperlactatemia(6).

1.4 The Pathophysiology of Septic Shock

The pathophysiology of septic shock is a complex host response to underlying infection associated with both systemic pro-inflammatory and anti-inflammatory responses. Sepsis occurs when the normal host response to an infection is dysregulated and leads to effects that are distant to the initial site of infection. The role of the early pro-inflammatory phase of sepsis, historically referred to as SIRS, is to target clearance of the infectious organisms through activation of the immune system. During this phase of illness, the typical response is an increase in total WBC count mainly due to increased neutrophils(16) and recruitment of neutrophils and other inflammatory mediators to sites of infection(17).

In addition to SIRS, there is a counter inflammatory response in sepsis known as the compensatory anti-inflammatory response syndrome (CARS) (18). The role of CARS is to deactivate the inflammatory system and restore normal homeostasis. This phase is characterized by immunosuppression with deactivation of granulocytes, lymphopenia and lymphocyte dysfunction(19). The host response to severe sepsis is outlined in **Figure 3**.
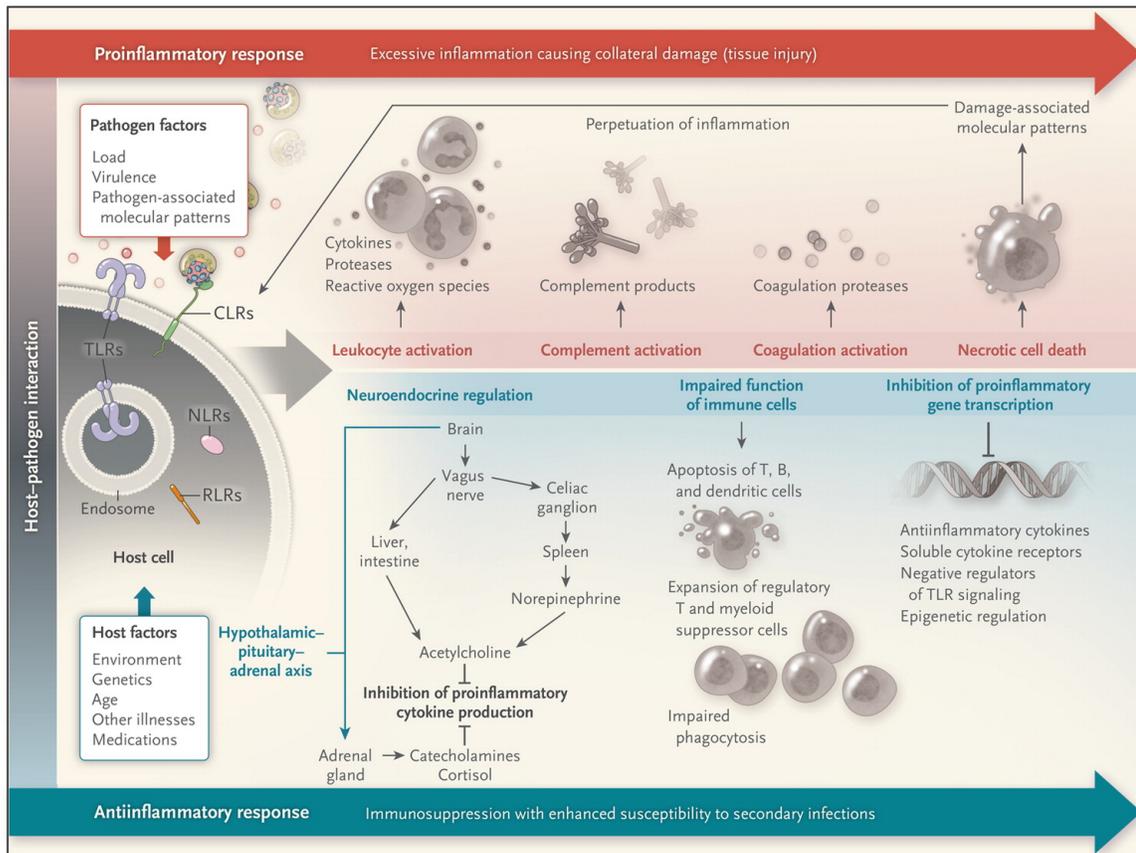
Figure 3. The Host Response in Severe Sepsis.

The host response to sepsis is characterized by both pro-inflammatory responses (top of panel, in

red) and anti-inflammatory immunosuppressive responses (bottom of panel, in blue). The

direction, extent, and duration of these reactions are determined by both host factors (e.g.,

genetic characteristics, age, coexisting illnesses, and medications) and pathogen factors (e.g.,

microbial load and virulence). Inflammatory responses are initiated by interaction between

pathogen-associated molecular patterns expressed by pathogens and pattern-recognition

receptors expressed by host cells at the cell surface (toll-like receptors [TLRs] and C-type lectin

receptors [CLRs]), in the endosome (TLRs), or in the cytoplasm (retinoic acid inducible gene 1–

like receptors [RLRs] and nucleotide-binding oligomerization domain–like receptors [NLRs]).

The consequence of exaggerated inflammation is collateral tissue damage and necrotic cell death, which results in the release of damage-associated molecular patterns, so-called danger molecules, that perpetuate inflammation at least in part by acting on the same pattern-recognition receptors that are triggered by pathogens.

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1.5 Outcomes of sepsis

Sepsis is the 2nd leading cause of non-cardiac ICU mortality and the tenth leading cause of death overall in the United States (2). Worldwide, infectious diseases are the third leading cause of preventable mortality(20). The mortality associated with septic shock was, historically, in excess of 80%, and despite advances in the care of critically ill patients with septic shock, it remains as high as 20 - 40% (6, 21, 22). In Canada between 2008 - 2009, sepsis represented 10.9% of hospital deaths with a crude mortality rate of 30.5%(7). Worldwide, sepsis may be the cause of up 5.3 million deaths per year(8). While overall mortality rates are useful when describing disease at a population level, tools to predict prognosis of individual patients in real-time are needed.

1.6 Prognostication

Traditionally, disease severity and prognosis in the ICU has been assessed using clinical scoring systems such as the Acute Physiology and Chronic Health Evaluation (APACHE) score, or the Sequential Organ Failure Assessment (SOFA)(23). The APACHE II score is made up of

12 physiologic and laboratory variables along with chronic health variables and gives a score up to a maximum of 71. The most abnormal value in first 24 hours of ICU admission is used, and the score incorporates age and chronic health problems, which are known to affect physiologic reserve. The APACHE II score has been shown to be associated with increased risk of death where those with the highest APACHE II score have the highest risk of death(24). Similarly, the SOFA score demonstrates illness severity as measured by organ dysfunction. The SOFA score is made up of a score between 0-4 (with increasing abnormality) of six different organ systems giving a possible score of 0-24. A SOFA score of greater than 15 is associated with a mortality rate of 90%(23, 25). Serial measurements in the SOFA score, and more specifically a rising SOFA score have been shown to associated with an increase in mortality in the ICU. An increase in the SOFA score during the first 48 hours of ICU admission predicts a mortality of over 50%(26).

Sepsis specific severity scores have been developed to estimate the probability of hospital mortality. The Sepsis Severity Score (SSS) is a score based on patient clinical characteristics and goal-directed therapies. The score ranges from 0 to 130 where higher values are associated with higher mortality(27). **Table 2** shows the components of the SSS.

These scoring systems are limited by the fact that they generally make assessments based on the first 24 hours of ICU admission as a predictor of outcomes, are cumbersome to use, and fail to consider clinical phenotypes, individual genotypes, response to treatment, and other factors.

Table 2: Sepsis Severity Score Mortality Prediction Model.

Reproduced from Osborn TM et al. Sepsis severity score: an internationally derived scoring system from the surviving sepsis campaign database*. *Crit Care Med.* 2014;42(9):1969-76 with permission from Elsevier.

Variable	Point Values
Sepsis origin	
Emergency department (ref)	0
Ward	10
ICU	11
Geographic region	
Europe	9
North America (ref)	0
South America	14
Other variables	
Cardiovascular organ failure (OF)	9
Lactate >4mmol/L	18
Cardiovascular OF with lactate >4	0
No hypotension (ref)	0
Unresponsive hypotension	10
Responsive hypotension	9
Received >=20 mL/kg of crystalloid or equivalent	1
Received vasopressors	9
Pneumonia	9
Urinary tract infection	5
Abdominal	8
Meningitis	2
Catheter	9

Device	10
Other infection	8
Renal OF	9
Hepatic OF	8
Hematology OF	10
No MV and no pulmonary OF (ref)	0
No MV and pulmonary OF	8
MV with plateau pressure <30cmH$_2$0 and no pulmonary OF	14
MV with plateau pressure <30cmH$_2$0 and pulmonary OF	14
MV with plateau pressure >30cmH$_2$0 independent of pulmonary OF	17
Hyperthermia (>38.3^{o}C or 101.0F)	2
Hypothermia (<36°C or 96.8F)	9
Chills with rigor	2
Tachypnea (>20 breaths per minute)	7
Leukopenia (WBC count <4 x 10e9/L)	9
Hyperglycemia (glucose >120 mg/dL)	4
Acutely alter mental status	8
Constant	

OF=organ failure; MV=mechanical ventilation; unresponsive hypotension= mean arterial pressure (MAP) <65 mmHg despite 20mL/kg crystalloid; responsive hypotension=MAP >65mmHg after 20mL/kg crystalloid

It is highly probable that septic shock, similar to other diseases like cancer or acute respiratory distress syndrome(28), represents several unique pathobiological states and a more "precision medicine" focus is required to accurately predict prognosis and the need for, or response to, treatment at different time points(29). The first step that could allow specific patients to be considered for different therapies at defined time-points in septic shock is to identify "like" groups(30). Several methods have been described to group similar cases, including analysis of inflammatory or metabolic biomarkers(31), genetic arrays (29) or assessing for similar trajectories within a population(30, 32). Although there is interest in identifying genetic characteristics that predict treatment response, to date no large studies have addressed this issue.

1.7 Group-based trajectory analysis

Group-based trajectory analysis is a statistical method that can be used to describe the pattern of a variable over time. The underlying principle is informed by the potential that unique subgroups within a population may follow distinct trajectories over time. Rather than pre-specifying groups within a population, or using modeling methods to measure an average trajectory for the entire population, group-based trajectory analysis allows for different groups with different trajectories to emerge from the data (33). This type of analysis lends itself well to data that evolves over time, such as the temporal trend of the complete blood count in a patient with septic shock.

1.8 Complete blood count

The complete blood count (CBC) is one of the most commonly ordered tests in medicine. The CBC provides information about red blood cells and hemoglobin concentration, the white blood cell (WBC) count and platelet count. Modern hematology analyzers report the total WBC count as well as a five-part differential. The five-part differential includes absolute and relative values of neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The normal range for the total WBC is generally considered to be 4.5 - 11 x 10e9/L. Leukopenia is defined as a total WBC count less than 4.5 x 10e9/L and leukocytosis is a total WBC greater than 11 x 10e9/L. Neutrophils comprise the largest proportion of total WBC, followed by lymphocytes, monocytes, eosinophils, and basophils.

The CBC is typically ordered at least daily in ICU patients, and perturbations in WBC count are common. White blood cells play an important role in the host response to sepsis. Evolving to neutralize pathogens and clear infection, components of WBC also directly contribute to the pathophysiology of sepsis.

1.9 WBC abnormalities in sepsis and trauma

Abnormalities of WBC count are commonly seen in critically ill patients. The WBC count is one component of the original SIRS criteria (WBC count <4 or >12 x 10e9/L)(5), and is incorporated in the APACHE II score as previously discussed (*Section 1.6 Prognostication*). WBC count abnormalities correlate with disease severity in the ICU(24). Marked abnormalities of the total WBC count contribute more points to the total APACHE II score than less severe

abnormalities (WBC >40 x 10e9/L or WBC <1 x 10e9/L each contribute 4 points)(24).

Although derangements in the total WBC count are associated with increased mortality in septic

shock, the specific changes in WBC over time in septic shock are not well described.

One small prospective study of 21 patients with septic shock showed that most patients had

a leukocytosis for the first 48 hours of hospital admission, primarily due to increased number of

neutrophils. In contrast, the lymphocyte component of the total WBC count was low and

remained low for the first 48 hours of ICU admission(16). One of the proposed mechanisms of

lymphopenia in septic shock is lymphocyte apoptosis, which likely contributes to the associated

immunosuppressed state(34). Persistently low lymphocyte counts have been shown to be

associated with increased illness severity, longer ICU length of stay as well as with poor patient

outcomes, including death(19). The small sample sizes of these studies limit the generalizability

of these results.

A small prospective observational study designed to evaluate neutrophil apoptosis in the

first 12 days of septic shock (n = 80 patients) showed that the mean leukocyte count was elevated

at 24 hours, 5 days and 12 days in both survivors and non-survivors(35). The leukocyte count at

day 12 was significantly higher in the non-survivors compared with survivors (mean 19.4 x

10e9/L vs. 13.8 x 10e9/L, p=0.04). This study only measured total WBC at 3 time points (24

hours, day 5 and day 12). An additional limitation of this study is the use of mean values for

total WBC rather than considering known variability and the potential for unique WBC count

trajectories of individual patients.

A retrospective study of 77 patients with sepsis or septic shock showed that survival is associated with total WBC count. In this study, 73% of surviving patients had neutrophil counts between 5 - 19 x10e9/L compared to only 42% of non-surviving patients. The authors also reported that 12% of survivors vs. 35% of non-survivors had a neutrophil counts between 0 – 4.9 x10e9/L(36). This study measured total WBC count and neutrophil count during the first 24 hours of hospital admission and provides information about the neutrophil count on the first day of sepsis but does not provide data on the WBC and neutrophil count over time.

A retrospective study of 2731 patients with septic shock, found that a low initial WBC count (<4 x 10e9/L) is associated with higher hospital and ICU mortality (odds ratio (OR) 1.85, 95% CI 1.38 to 2.48). This effect was independent of other factors including age, baseline creatinine, baseline bilirubin, blood pressure, heart rate, respiratory rate, chronic kidney disease, number of organ failures, and NYHA class IV heart failure(37). Similar to the aforementioned study, evaluating the baseline WBC count fails to consider the impact of WBC changes over time.

A large retrospective study of 2448 trauma patients showed that a failure to normalize leukocytosis as well as a failure to normalize lymphopenia were both associated with increased risk of death at 20 days(38). This study evaluated all CBC results measured in the first 96 hours of hospitalization following trauma. The study authors separated patients into 3 groups based on total WBC count, neutrophil count and lymphocyte count. The 3 groups for total WBC count were: (1) initial leukocytosis, return to normal, (2) initial leukocytosis, no return to normal (persistent leukocytosis), and (3) no leukocytosis. Similar groups were made for the neutrophil

and lymphocyte count. This study showed that persistent leukocytosis was associated with a 2.5-fold increase in the risk of death (95% CI 1.47 to 4.24). This study did not find an association between risk of death and either neutrophilia that returned to normal or persistent neutrophilia which is unusual since total WBC count is made up primarily of neutrophils. Persistent lymphopenia was associated with a 1.64 fold increase in the risk of death (95% CI 1.02 to 2.64)(38). A limitation to this study is that the authors predefined the groups of patients and fit individuals into these groups, rather than letting the data guide group definitions and between-group interactions.

1.10 Limitations of existing literature

The main weakness of all prior studies that sought to associate the time course of the WBC count with outcomes in critical illnesses is that their metric evaluated the time course of the *average* WBC in the entire population under study. Implicit in this is that the overall average meaningfully represents all individuals. The results of such a strategy would be misleading if, as seems likely, there are phenotypically disparate subsets of patients whose WBC counts take on characteristically different time courses. Indeed, the overall average may not be representative of any individuals in the population. Such disparate time courses could plausibly reflect differences in genetics, pathogens, comorbid conditions, environment, or interactions of any of these elements. In addition to this limitation, the studies presented above were small, dated and do not reflect current standards of care in septic shock. Because the WBC count is simple to order and interpret and is ubiquitously available in ICUs, the relevance of the WBC count, or more precisely the WBC count trajectory, should be re-evaluated. In my thesis work I have attempted to avoid the shortcomings of using average WBC count values by using trajectory

analysis, a statistical technique that identifies subsets of individuals who follow a similar

progression over time.

CHAPTER 2 – OBJECTIVES AND HYPOTHESES

2.1 Study Objectives and Hypotheses

The purpose of my thesis is to evaluate trajectories of the WBC count in patients with septic shock, and to evaluate the association of WBC count trajectories with hospital mortality in this vulnerable patient population. My hypothesis is that there are distinct groups of individuals with septic shock, perhaps with different disease biology, or response to treatment that can be identified using trajectory analysis of their WBC counts. I further hypothesize that the identified trajectories will be able to predict mortality.

My specific objectives are as follows:

1) Use trajectory analysis to identify subgroups of patients with septic shock with differing patterns of WBC counts over time.

2) Identify characteristics of patients and pathogens associated with the unique WBC count trajectories identified.

3) Evaluate the association between the unique WBC count trajectories and 30-day mortality in patients with septic shock.

CHAPTER 3 - METHODS

3.1 Research Design, and Study Population

This is a two-center, retrospective cohort study. We used a large existing clinical septic shock database to identify patients ≥18 years of age with septic shock who were admitted to an ICU at either the Health Sciences Centre or the Saint Boniface Hospital, two tertiary hospitals in Winnipeg, Canada, between the years of 2006 and 2012.

Inclusion criteria for this study are:

1) Age >18

2) Vasopressor-dependent septic shock

3) ICU length of stay >48 hours

4) At least 3 WBC measurements while in ICU

Exclusion criteria are:

1) Malignancy, HIV, cirrhosis, neutropenia or immunosuppression including chemotherapy as co-morbidities

2) Re-admission to the ICU for septic shock within the specified study period

Patients with malignancy, cirrhosis, HIV or immunosuppression listed as co-morbidities in the Cooperative Antimicrobial Therapy of Septic Shock (CATSS) database were excluded

from this study to limit cases with abnormalities of the WBC due to alternative diagnoses. We limited our analysis to those patients who have an ICU length of stay of at least 48 hours to mitigate survival bias and at least 3 WBC measurements to be able to determine trajectory. For subjects with more than one ICU admission for septic shock, the first admission was included in the analysis.

3.2 Data Source

The data for this study was derived from a pre-existing database that has been developed by combining the CATSS database, and laboratory variables from the computerized Laboratory Information System from the Health Sciences Centre and the Saint Boniface Hospital.

The CATSS database includes patients admitted with septic shock from 30 centers in Canada (including the two study centers), the United States and Saudi Arabia. Each institution contributed a minimum of 50 cases. Trained research personnel maintain the database using a standardized and piloted data extraction template. Each case is screened to ensure a standardized definition of septic shock was utilized, as defined by the 1991 SCCM/ACCP Consensus Statement on Septic Shock(5). All patients are required to exhibit vasopressor-dependent septic shock, and all patients with mixed shock states are excluded. Data are collected on individual patients until time of death, or hospital discharge, up to 30 days. The CATSS database is the largest reported clinical septic shock database and the source of numerous publications on septic shock(39, 40).

The CBC data was obtained from the computerized laboratory information system at St. Boniface Hospital and Health Sciences Centre. CBC results were obtained from a Sysmex XE-2100 automated hematology analyzer (Sysmex Canada, Mississauga, Canada). WBC measurements from this analyzer are exceptionally precise with minimal carryover between samples. The coefficient of variation for the WBC is <2.0% and the high-to-low carryover testing was 0% for WBC(41) indicating that the Sysmex XE-2100 measures the total WBC and differential with a high degree of precision and accuracy.

Members of our research group have previously linked the databases that was used for this thesis. Linking of the databases was completed using last name, date of birth, personal health information number, and CATSS database study number. This database has previously been permanently anonymized for secondary data analysis.

3.3 Study Variables

Variables that are collected in the CATSS database include: age, sex, geographic location, APACHE II score(24), number of new organ failures on day 1 of ICU admission, type of organ failure (cardiovascular, renal, respiratory, metabolic, or hematologic), comorbid conditions (liver failure, chronic obstructive pulmonary disease (COPD), diabetes, New York Heart Association (NYHA) class IV heart failure, dialysis dependency, malignancy, human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS), immunosuppression); documented or presumed site and source of infection; supportive treatments (e.g. Mechanical ventilation, vasopressors/inotropes, renal replacement therapy, time to first appropriate antimicrobial, and the provision and timing of appropriate or combination

antimicrobial agents). Organ failures were defined as follows: Cardiovascular = systolic blood

pressure <90mmHg or more than 40mmHg drop from normal OR MAP <65 mmHg for at least

one hour despite adequate fluid resuscitation (2L saline or equivalent) OR the use of

vasopressors; Renal = elevation of normal baseline creatinine to >1.5 x normal value;

Respiratory = ventilation required; Hematologic = platelet count <80 x 10 e9/L; Metabolic =

lactate >3mmol/L.   Appropriate antimicrobial therapy was defined by administration of

antimicrobials with *in vitro* activity for the isolated pathogen or appropriate for the clinical

syndrome in cases where no pathogen was isolated(42). Combination antibiotic therapy was

defined as concomitant use of two or more appropriate, intravenous, bactericidal antibiotics with

different mechanisms of action for at least 24 hours after the onset of hypotension(39).

Laboratory variables collected include CBC results (WBC and differential, hemoglobin, platelet

count), INR, bilirubin, creatinine, and lactate. The CATSS database also includes ICU survival,

30-day survival, ICU length of stay.

A new variable called APACHE II*WBC was created to reflect the APACHE II without the

influence of the initial WBC count. This variable is calculated in the following way:

a. If WBC >=40 or <= 1, then subtract 4 from APACHE II score

b. If WBC 20-39.9 or 1-2.9, then subtract 2 from APACHE II score

c. If WBC 15-19.9, then subtract 1 from APACHE II score

d. If WBC 3-14.9, then do not alter the APACHE II score

A complete list of variables is presented in **Table 3**.

Table 3: Complete list of variables included in analyses

Variable	Description
Patient variables	
Sex	Categorical
Age	Continuous
Liver failure	Categorical
Chronic obstructive pulmonary disease	Categorical
Diabetes mellitus	Categorical
Chronic Kidney Disease	Categorical
Dialysis dependence	Categorical
NYHA Class IV	Categorical
Hospital	Categorical
Illness variables	
Duration of hospitalization before shock (days)	Continuous
APACHE II score	Continuous
Platelets (x10e9/L)	Continuous
Serum creatinine (umol/L)	Continuous
INR	Continuous
Bilirubin (umol/L)	Continuous
HCO_3 (meq/L)	Continuous
Total number of day one organ failures	Continuous
Renal failure*	Categorical
Hematologic failure*	Categorical

Hepatic failure*	Categorical
Metabolic failure*	Categorical
Respiratory failure*	Categorical
Pathogen Variables	
Culture positive	Categorical
Bacteremia	Categorical
Site of infection	Categorical
Primary organism	Categorical
Co-intervention variables	
Time to 1st antibiotic (hrs)	Continuous
Combination antibiotic use	Categorical
Appropriate antibiotic use	Categorical
Surgical source control	Categorical
Mechanical ventilation	Categorical
Hemodynamic support	Categorical
Outcome variables	
30-day survival	Categorical
ICU survival	Categorical

NYHA=New York Heart Association; APACHE II=Acute Physiology and Chronic Health Evaluation II; INR=International Normalized Ratio; HCO3=bicarbonate
*Organ Failure definitions: Renal = elevation of normal baseline creatinine to >1.5 x normal value; Respiratory = ventilation required; Hematologic = platelet count <80 x 10 e9/L; Metabolic = lactate >3mmol/L

3.4 Statistical Analyses

3.4.1 Objective 1 – Assess the number and time course of the different trajectories of WBC counts in patients with septic shock using trajectory analysis

Background on Group-Based Trajectory Analysis

We used group-based trajectory analysis to determine the trajectory of the total WBC count. Trajectory analysis is a statistical method used to describe the course of a trend over time(43). Proc TRAJ is a SAS procedure that can identify groups of individuals with similar characteristics over time using group-based modeling (33, 43, 44). Proc TRAJ is available as a SAS add-on from http://www.andrew.cmu.edu/user/bjones/. Proc TRAJ allows for trajectories to be individually fitted to polynomials up to fifth order(44). Our assumption was that heterogeneity exists in the WBC count over time and that there are distinct groups of patients that follow a similar trajectory (eg. Start high, stay high; start high, normalize; start normal, fall below normal; start low, normalize; start low, stay low) and therefore trajectory analysis is an appropriate method to more completely assess the WBC count in septic shock. We sought to identify the optimal number of groups exhibiting distinct trajectories of total WBC count. The Proc TRAJ algorithm can fit any number of groups, so to choose the optimal number of groups, we considered the overall degree of fit for each solution based on the Bayesian Information Criterion (BIC) (see below), while simultaneously considering whether the different trajectories appeared to separate to a clinically relevant degree. Graphical representation of the fitted trajectories, in this case the WBC count trajectory over time for each group in patients with septic shock, is a standard output from Proc TRAJ.

Mock data set-up for Proc TRAJ and explanation of syntax

In this section, an example of the data set-up and syntax to run Proc TRAJ are explained. To analyze data using Proc TRAJ, multiple measurements of the variable of interest are required and the data must be organized into a wide format. Data was formatted such that each subject has only one row of data with multiple observations. An example of a mock data set is included in **Table 4**.

Table 4: Mock WBC trajectory data set for Proc TRAJ

Subject	T_1	WBC_1	T_2	WBC_2	T_n	WBC_n	APACHE II	DEAD (0=yes)
1	1	15.2	2	14.2	3	8	24	0
2	1	9.7	2	15.6	3	.	20	1
3	1	20.2	2	18.4	3	13.2	22	1
4	1	6.4	2	5.7	3	.	20	0

. Denotes missing data

An example of the syntax and explanation for the components for Proc TRAJ are included below.

Proc TRAJ data=wbctrajectory out=OF outplot=OP outstat=OS;

Var **WBC1-WBCN**; /* Variable of Interest */

Indep **T1-TN**; /* Time Variables */

Model **CNORM**; /* Censored Normal Model */

Min **0**; /* Lower Censoring Point */

Max **200**; /* Upper Censoring Point */

Ngroup **3**; /* Fit 3 Groups */

Order **3 3 3**; /* Cubic Trajectory for Each Group */

Dropout **2 2 2;** /* probability of drop out depending on the previous 2 results*/

Obsmar **dead 0 0**; /*denotes observations which are missing at random*/

Run;

The var statement refers to the variable of interest (in this case WBC count), measured at different times. Indep refers to when the dependent variables are measure (time). Model refers to the probability distribution for the dependent variable (WBC) which can be truncated normal (cnorm), poisson (zip) or logistic (logit). Min and max are required for the cnorm model and denote the minimum and maximum values for the dependent variable. The censored normal model for Proc TRAJ was used as for the WBC count trajectory for septic shock as it was assumed to follow an approximately normal distribution. The distribution for total WBC count cannot be normally distributed with a zero as the lowest value and values that extend upward, but this data is approximately normal so the cnorm model is appropriate. Ngroup tells Proc TRAJ the number of trajectories to model. Order is degree of polynomial for each group and can be 1st order which will fit a linear line, 2nd order which will fit a parabolic form, 3rd order which fit a curve with 2 inflection points and so on up to 5th order. An optional dropout model can be incorporated into Proc TRAJ which includes the probability of dropout which can be modelled with 0=constant rate, 1=depends on previous response, or 2=depends on the two previous responses for each group. Within the dropout model, you can tell Proc TRAJ which observations

are to be included in the dropout model and those to be treated as missing at random

(1=observations missing at random and 0=observations to be modeled in dropout).

Model Building

The database was reformatted into the format appropriate for Proc TRAJ. To maximize

the fidelity of the analysis, we initially included up to 4 WBC measurements per day (one from

each 6-hour period starting at 0h00 and ending at 23h59). In the event that a subject had more

than one CBC per quarter day, the first WBC count from that time period was included. This

approach however, resulted in a large proportion of intervals with no data so we reduced the

analysis to include up to 2 measurements per day (the first in each 12-hour period). As the

precise time of admission to ICU is not captured in the CATSS database, we anchored the start

of the trajectory analysis to the time of the first CBC drawn, as the majority of patients will have

a CBC drawn at or shortly after the time of ICU admission. We included measurements for the

first 7 days after ICU admission to capture the WBC trajectory that is attributable to septic

shock. The median duration of ICU admission in this database in 7 days.

We followed a standard and previously described procedure for model building using

Proc TRAJ(44). Model building started with fitting one group, then two groups, and then three

groups and so on. Model selection was performed using both clinical assessment and a standard

method of evaluating the change in the Bayesian Information Criterion (BIC) between two

models(43). ΔBIC = BIC complex – BIC null. The complex model is the model with the higher

number of groups and/or higher degree of polynomial, whereas the null model is the simpler of

the two models being compared.   The BIC output from Proc TRAJ is based on the following

formula:

      BIC = log (L) – log(N)*0.5k

      Where L = the maximum likelihood from the model

         N = sample size

         k = the number of parameters

In this case BIC is always a negative number. The first component of the BIC calculation

measures the improvement in model fit based on increasing the number of parameters, while the

second component applies a penalty for increasing the complexity of the model. The logged

Bayes factor (2*ΔBIC) provides evidence against H$_o$ (simpler model)(44) and a table showing

the interpretation is shown in **Table 5**(43, 44). Model comparison was done in a step-wise

manner such that the two-group model is compared to one-group, and the three-group model is

compared to the two-group model and so on.

Table 5: How to interpret 2*(ΔBIC)

2*(ΔBIC)	Evidence against H_o
0 to 2	Not worth mentioning
2 to 6	Positive
6 to 10	Strong
>10	Very strong

The BIC output from Proc TRAJ includes one value for BIC *observations* and one value for BIC *subjects*. The BIC *observations* reflect the BIC value for all the multiple observations for each subject, whereas the BIC *subjects* reflect the BIC for the total number of subjects included in the analysis. The BIC *observations* overestimates the true N of the sample due to the intra-individual observations not being completely independent and the BIC *subjects* underestimates the true N due to fact that there is some independence in intra-individual variation, thus the true BIC for the model is somewhere between these 2 values(33).

We constructed various models starting at 1 group, up to 7 groups which was the point where the BIC was no longer significantly better and, the trajectory groups were small and no longer clinically relevant. We reviewed the graphical outputs for face-validity in the clinical setting, and both the BIC as well as the change in BIC, to provide statistical evidence in support of group choice. For this stage of model building we set the degree of polynomial to 3rd order. A 3rd order polynomial was chosen because the cubic function is the simplest representation that can fit a relatively complex WBC trajectory, such as a rise, fall, and subsequent rise again in WBC count. To account for subjects who are missing data due to death before 7 days, and to

distinguish these from subjects who simply did not have a blood sample collected in a given 12-hour period, a dropout function was included in Proc TRAJ. This drop out function allows one to include a logistic model of dropout probability per wave with 0 = constant rate, 1 = depends on the previous response, 2 = depends on the two previous responses, for each group. We coded the probability of drop out to be based on the 2 previous responses in order to incorporate more information into the mathematical model. I also included the 'Obsmar' function in Proc TRAJ which is an optional binary variable to mark which observations are to be included in the dropout model and those to be treated as missing at random. This variable = 1 for observations to be treated as data missing at random and = 0 for observations to be used for the modeled dropout (i.e. Deaths). In this way, we are able to show which data are missing due to death.

After several iterations of Proc TRAJ, the 6-group trajectory model showed a singular convergence error. A singular convergence error means that the automated starting values imputed by Proc TRAJ failed to provide accurate model estimations, and manual input of the starting value for each trajectory is required.

Once the number of trajectory groups was chosen, the degree of polynomial was changed from 1 to 5 to determine the best overall fit. We pre-specified 3rd order polynomial to represent the WBC trajectory *a priori* so only considered changes if the BIC or graphical representation was felt to be significantly improved.

Model Diagnostics

We conducted an assessment of the final trajectory model to determine the quality of the model's fit to the data. Model fit parameters tested included an assessment of: 1) the ratio between the probability of group membership to the actual group assignment, 2) the average posterior probability (AvePP) of correct group assignment, and 3) the odds of correct classification (OCC) statistic(33).

The ratio between probability of group membership (πj), which is the output shown in the trajectory plot, and the actual number assigned to each group should be close to 1. The AvePP is calculated by taking the average of all the posterior probabilities for a given group. The posterior probability is the probability that an individual with a specific pattern belongs to a specific trajectory group. A rule of thumb for assessing model fit using the posterior probability is that each group should have an AvePP of at least 0.7 (33). When using the OCC statistic, the higher the value is the better the model fit. OCC is calculated using the following formula:

$$OCC_j = \frac{AvePP_j/(1 - AvePP_j)}{\pi_j/(1 - \pi_j)}$$

Where OCC_j = the odds of correct classification into group j

             $AvePP_j$ = the average posterior probability of group j

             π_j = estimated population base rate of assignment into group or

probability of group membership (standard output from Proc TRAJ).

3.4.2 Objective 2 - Associations between identified WBC trajectories and patient demographics, comorbidities, and pathogen characteristics.

We summarized and presented the baseline characteristic of the entire cohort and then compared the baseline characteristics in the groups determined by trajectory analysis. We presented summary statistics as means with standard deviation or medians and 25th and 75th quartiles for continuous variables, and frequencies and proportions for categorical variables. Because more than 2 trajectories are present, we compared baseline characteristics using ANOVA for continuous variables that were normally distributed or the Kruskal-Wallis test for continuous variables that were not normally distributed and the Chi-square test for categorical variables.

We used a multinomial logistic regression model (Proc LOGISTIC) to assess the association of independent variables and the trajectories identified in Objective 1. This model demonstrates the association of patient, illness and pathogen characteristics with a given trajectory compared to the reference group. Trajectory 1 was chosen as the reference group because it was the largest group and was the group with the lowest WBC count. Model building started with univariable multinomial regression models to assess variables associated with the different trajectories. A multivariable multinomial regression model was then built using pre-specified variables and included age, sex, APACHE II score modified to exclude the effect of baseline WBC count (APACHEII*WBC), bacteremia, time to antibiotics (hours), use of appropriate antibiotics, number of organ failures at time of ICU admission and baseline platelet count. These variables were included in the model regardless of p-value on univariable testing because all variables are deemed to be of clinical significance. We reported the odds ratio, and

confidence intervals, for all clinically important independent variables. We did not perform tests

for model goodness-of-fit because we included all variables regardless of p-value on univariable

modeling.

3.4.3 Objective 3 - Association between WBC trajectories and 30-day mortality and the duration of use of shock requiring vasopressor support

Unadjusted ICU and 30-day mortality estimates were made based on each trajectory

group and compared using the Chi-square test.

To investigate the independent association of WBC trajectories on 30-day mortality, we

constructed a multivariable Cox proportional hazard models (Proc PHREG) adjusted for known

or presumed variables expected to influence mortality. A univariable Cox proportional hazard

model was initially constructed to demonstrate the unadjusted mortality associated with each

trajectory group. The variables that were tested on univariable analysis include: age, sex,

APACHE II score, number of organ failures on day 1, time to first appropriate antibiotic (hours),

appropriate antibiotics use, co-morbidities (COPD, diabetes mellitus, chronic kidney disease,

dialysis dependence, NYHA class IV heart failure, baseline platelet count, baseline creatinine,

culture positive, bacteremia, type of infection (catheter-related infection, respiratory, urinary

tract, intra-abdominal, CNS, skin and soft tissue, surgical site, cardiac pericardial, or other), gram

positive, gram negative, fungal, or other.

Building on the univariable analysis, I developed the multivariable Cox proportional

hazard model which included variables known or presumed to be related to mortality in septic

shock. Variables included in this analysis were patient variables (age, sex), illness variables (APACHE II score using the modified APACHE II*WBC variable, number of organ failures on day 1 of ICU admission), pathogen variables (bacteremia, culture positive or negative,) and co-intervention variables (time to first antibiotic, the provision of appropriate, or combination antibiotics). The admission time to ICU was used to denote time zero in the Cox regression model and survival data was collected until time of death or to a maximum of 30-days or hospital discharge. No additional censoring was included. All variables were included in the model at time zero. With a sample size of 917 patients, and a mortality rate of 26%, we assumed that a model with up to 20 degrees of freedom could be used without overfitting(45). Finally, a Kaplan-Meier survival curve was plotted and the survival curves were compared using the log-rank test.

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). A p-value of <0.05 was considered to be statistically significant.

3.5 Ethics

This study was approved by the University of Manitoba Health Research Ethics Board on August 22, 2017 (HS21067, H2017:270). The database to be used for this study has been previously linked as part of a separate study with approval from Research Ethics Board at the University of Manitoba under the CATSS database study (Principle Investigator Dr. Anand Kumar). There is no identifying information in the database and no ethical concerns are anticipated. Informed consent is not required. All data files were secured with password

protection on a secure server in a locked office. Access was granted only to members of the

research team.

CHAPTER 4 - RESULTS

In this chapter, I will review the results of the trajectory analysis (Objective 1), and demonstrate the procedures used to arrive at the final model for total WBC count. I will then describe the clinical features associated with WBC trajectory as outlined in Objective 2, and the outcomes associated with different WBC trajectory as defined in Objective 3.

We identified 1758 patients with septic shock treated at the two study centers between 2007 and 2012. 841 patients were excluded for a final study cohort of 917 patients. To exclude secondary causes of WBC count abnormalities, 323 patients with malignancy, 55 with chemotherapy or immunosuppression, 3 with known or pre-existing neutropenia, and 84 with cirrhosis were excluded. We further excluded 19 with mixed-shock states. Finally, we excluded 1 patient who was re-admitted, 152 records with un-linkable data and 205 patients with fewer than 3 WBC measurements. The flow chart for cohort development is shown in **Figure 4**.
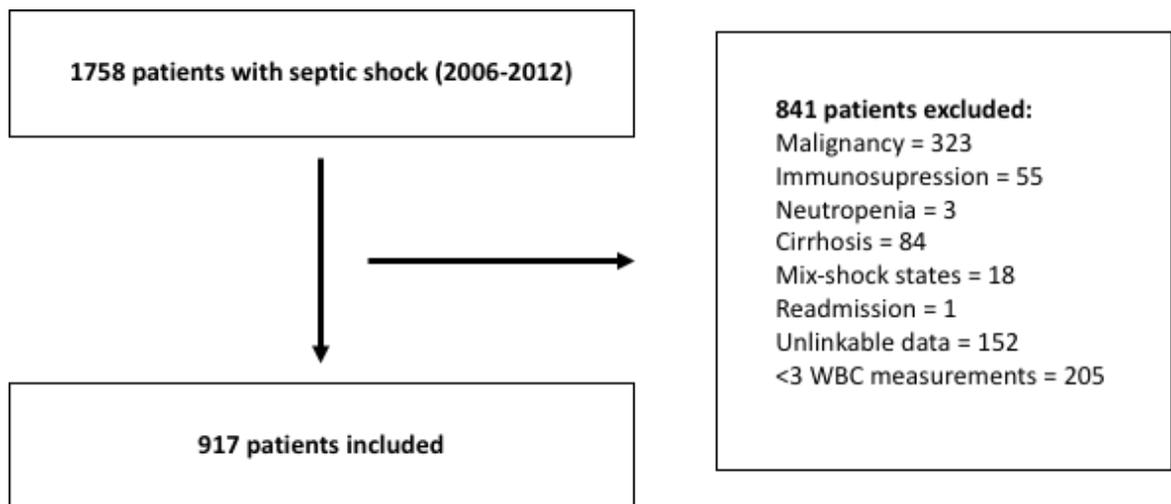
Figure 4: Flow diagram of study cohort development

4.1 Objective 1 - Number and time course of the different trajectories of WBC counts in patients with septic shock using trajectory analysis

4.1.1 Trajectory development and assessment of fit using 2 measurements per day with time zero anchored to time of first WBC collection

Assessing trajectory fit based on graphical output from Proc TRAJ

To start trajectory model building using Proc TRAJ, I set the order of polynomial to 3rd order and ran the procedure starting with 1 group, increasing to 7 groups. The graphical output provided by Proc TRAJ for groups 1 – 7 are outlined below in **Figures 5 - 13**. As the number of groups increased, we observed the emergence of a small group (less than 2% of the total population) with a distinct trajectory starting at 3 groups. This small group continued to be observed all the way through up to 7 groups. This identified a group with a significantly higher WBC count than the other groups. All 7 trajectory model groups are shown in **Figure 5**. A more detailed description of each trajectory model will follow.
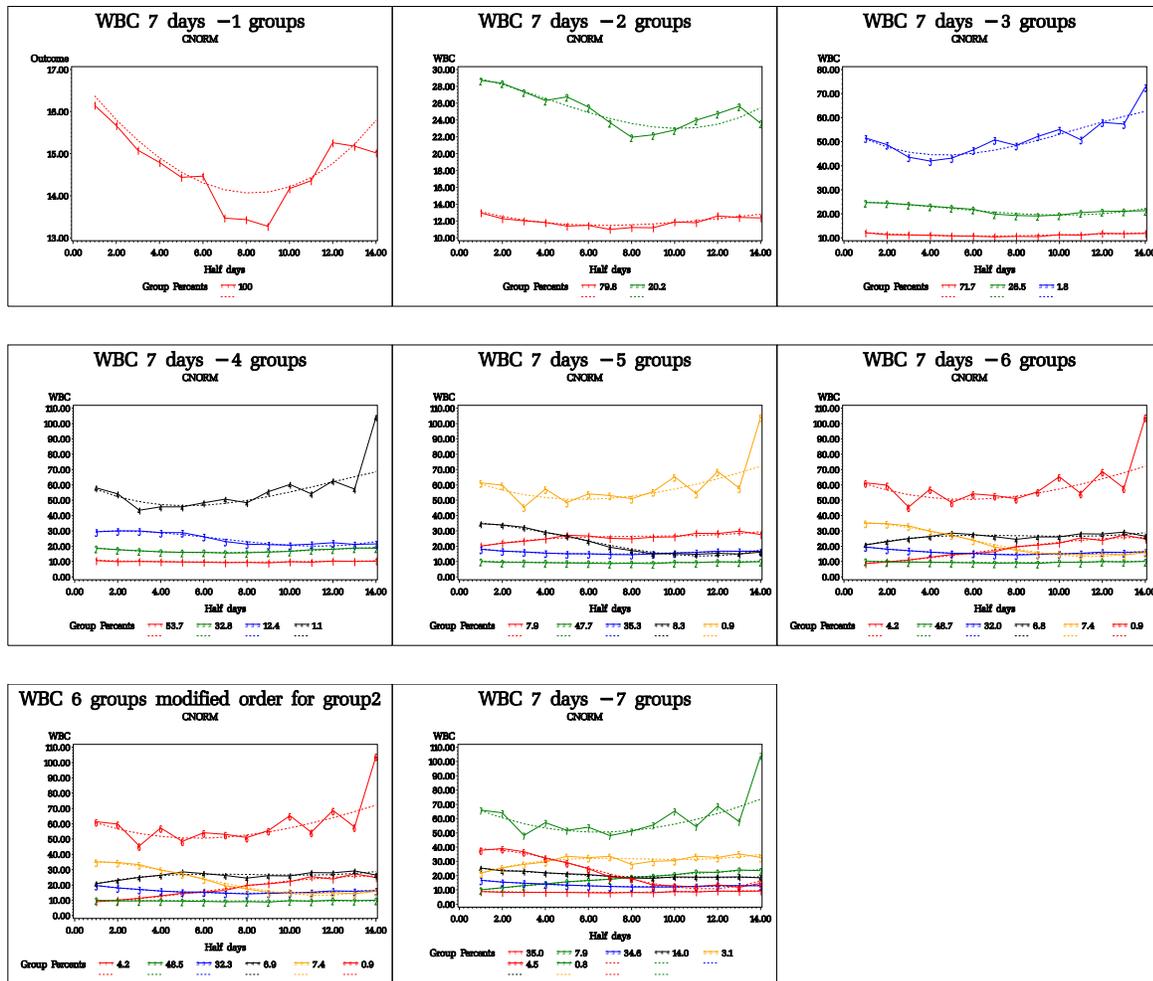
Figure 5: Graphical output of WBC count trajectory model building using Proc TRAJ from 1 group to 7 groups

Using a model that fits everyone into one group (see **Figure 6**), we can observe that on average the WBC count starts high (approximately 16 x 10e9/L), decreases over the first 4 days, and then rises again by day 5 to 6.
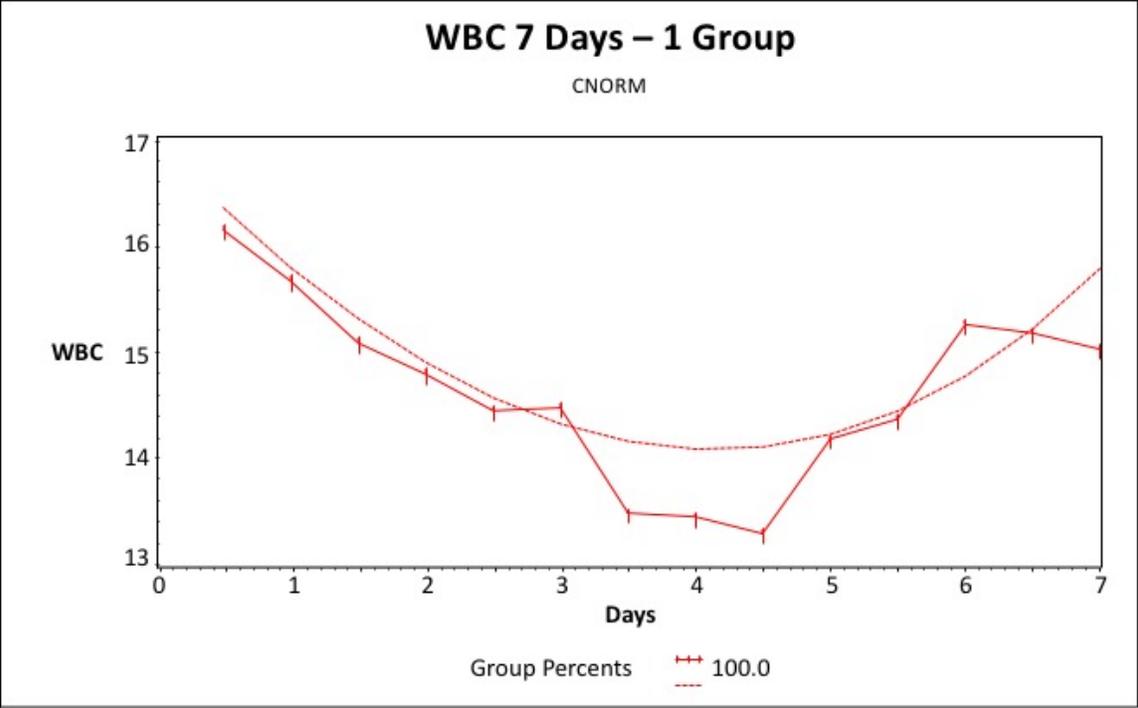
Figure 6: Graphical representation of the 1-group model from Proc TRAJ

This plot is observing the trajectory of model using 1 groups, incorporating WBC data for 7 days after admission to ICU

The 2-group model identifies 1) a group that starts with a slightly elevated WBC that gradually decreases and rises again near the end of the trajectory. This group represents the vast majority of the population (80%) and 2) a group with a WBC count that starts high, decreases and then starts to rise by day 5 to 6 representing 20% of the population This model is shown in **Figure 7**.
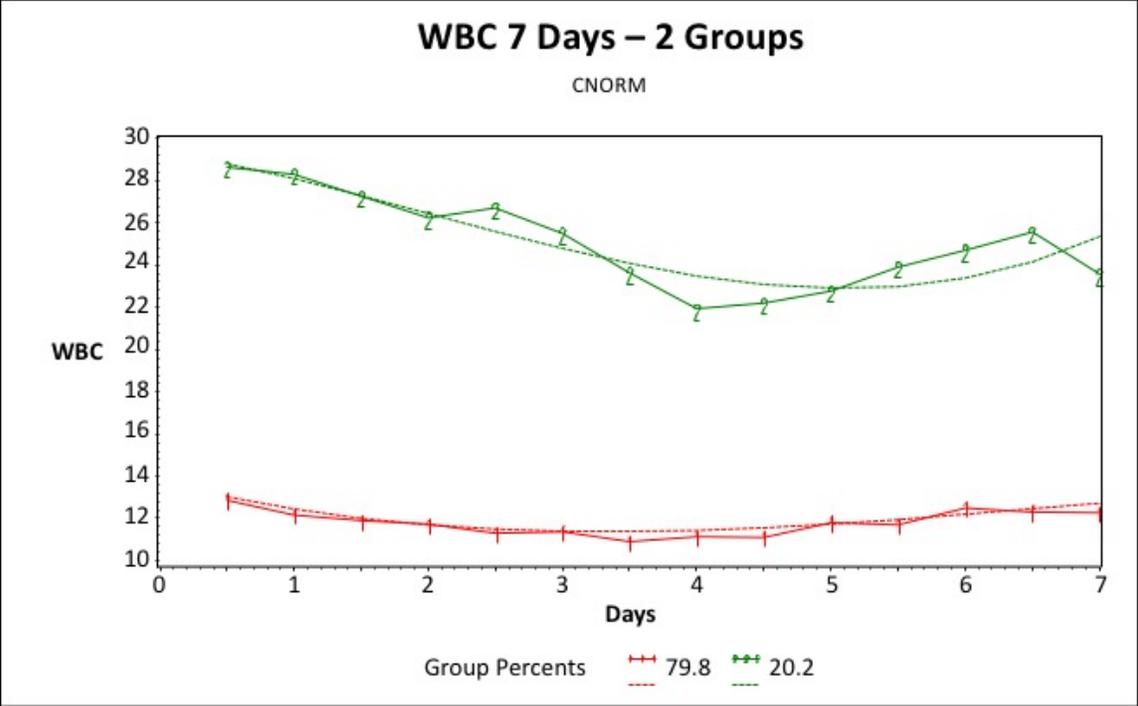
Figure 7: Graphical representation of the 2-group model from Proc TRAJ
This plot is observing the trajectory of model using 2 groups, incorporating WBC data for 7 days after admission to ICU

The 3-group trajectory model splits the population into 1) a large group (72%) which follows a fairly flat trajectory with a WBC count starting around 12 x 10e9/L, 2) a smaller group made up of 27% of the population that starts high and gradually decreases, and 3) a very small

group with 2% of the population with a significantly elevated WBC count. The 3-group model is

shown in **Figure 8**.
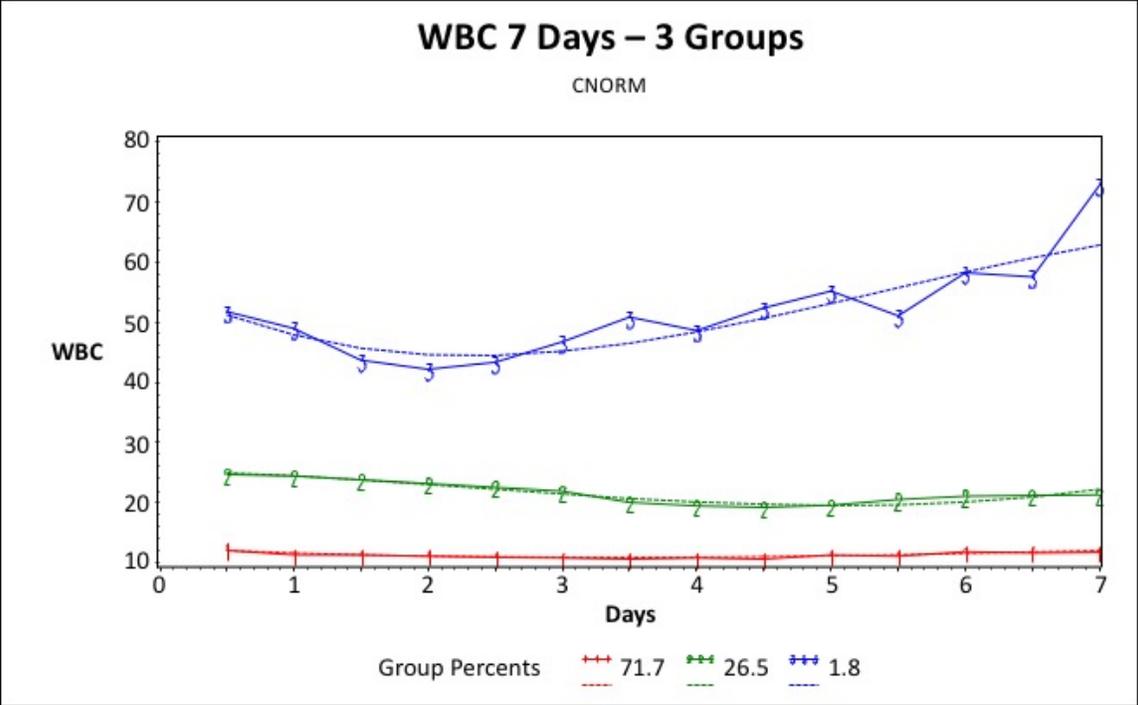
Figure 8: Graphical representation of the 3-group model from Proc TRAJ

This plot is observing the trajectory of model using 3 groups, incorporating WBC data for 7 days

after admission to ICU. This is the first observation of an outlier group with less than 2% of the

population with a significantly elevated WBC count

The 4-group trajectory model shows a similar flat trajectory group (54%) and small group with a significantly elevated WBC count group (1%) as the 3-group model. The middle trajectory from the 3-group trajectory model splits into 2 groups with a high WBC that comes down over time (12%) and a flat trajectory with a WBC that is elevate to about 20 x 10e9/L (33%). The 4-group trajectory model is shown in **Figure 9.**
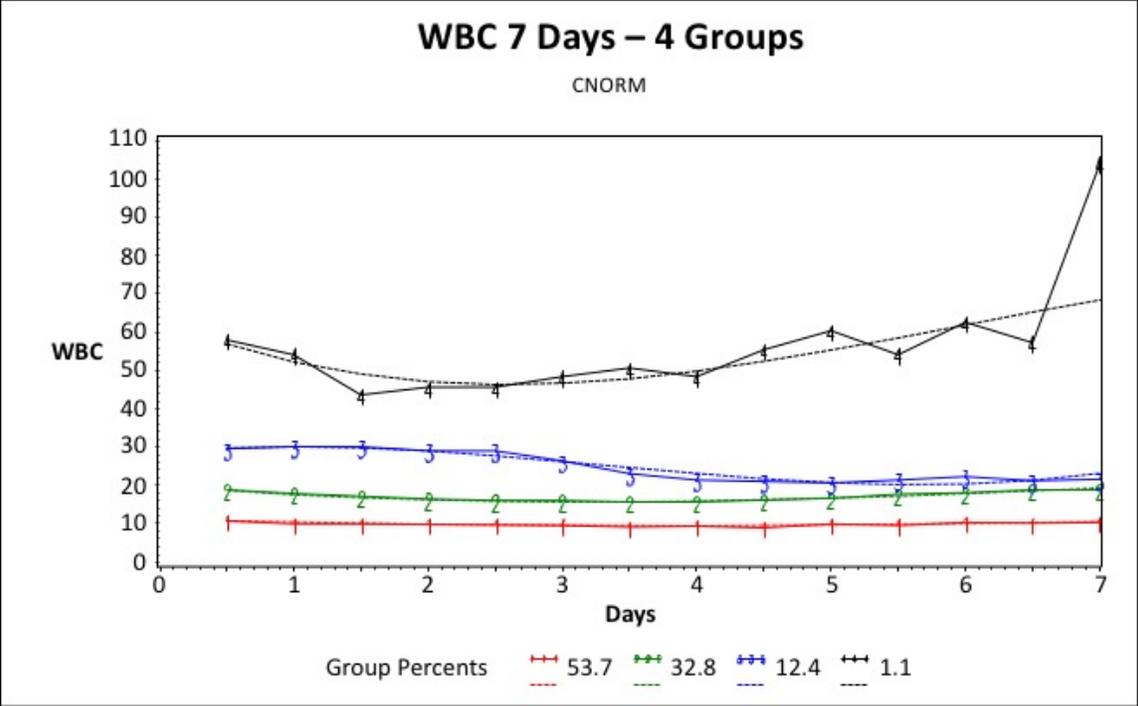
Figure 9: Graphical representation of the 4-group model from Proc TRAJ

This plot is observing the trajectory of model using 4 groups, incorporating WBC data for 7 days after admission to ICU

When fitting a 5-group trajectory model, as seen in **Figure 10**, the same flat normal WBC trajectory (Group 2 - 48%) and significantly elevated WBC trajectory (Group 5 - 1%) are again seen. The 5-group model identifies 3 other groups with different trajectories, one that starts high and normalizes (Group 4 - 8%), one that starts high and continues to rise (Group 1 - 8%), and one that stays relatively flat but at a higher baseline WBC count (Group 3 - 35%).
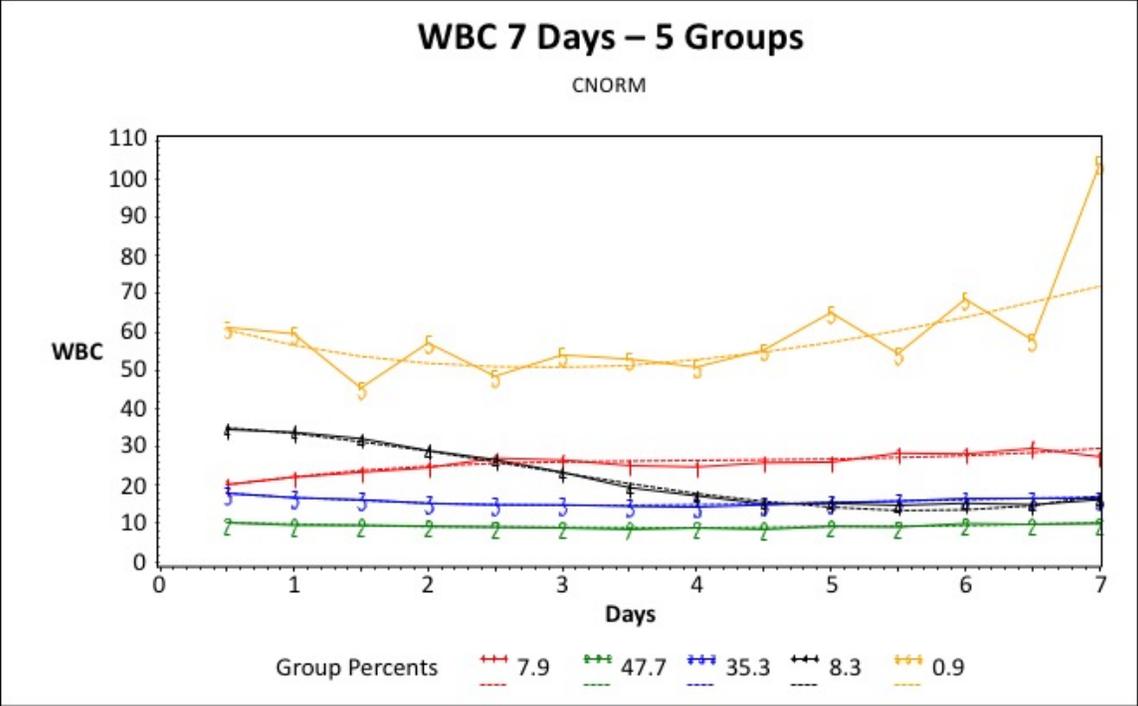
Figure 10: Graphical representation of the 5-group model from Proc TRAJ

This plot is observing the trajectory of model using 5 groups, incorporating WBC data for 7 days after admission to ICU

When fitting a 6-group trajectory model as shown in **Figure 11**, we see group 1 which starts normal and steadily increases (4%), group 2 which starts normal and follows a flat trajectory (49%), group 3 which starts high and decreases slightly but always remains above

normal (32%), group 4 which starts high and increases over the first 2 to 3 days and then

plateaus (7%), group 5 which starts very high and steadily decreases (7%), and group 6 which

has a significantly elevated high WBC count (1%). The 6-group model produced a singular

convergence error with the automated Proc TRAJ function.
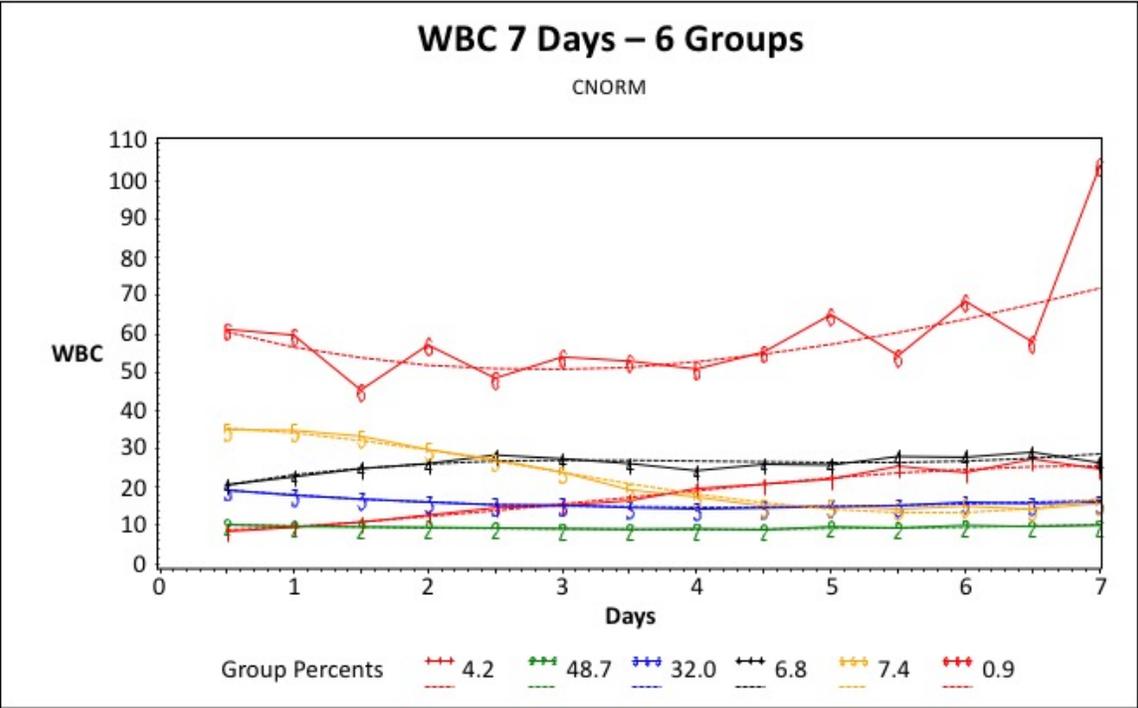
Figure 11: Graphical representation of the 6-group model from Proc TRAJ

This plot is observing the trajectory of model using 6 groups, incorporating WBC data for 7 days

after admission to ICU. Note this model had a singular convergence error in Proc TRAJ.

To address the issue of model convergence I reviewed the output contained within the

SAS log. A convergence error occurs when the automated starting values determined within

Proc TRAJ do not work and manual input of these values is required. In the SAS log file, the

parameter estimates for the 6-group trajectory model are contained within the output. I copied

the values into the SAS code to manually direct Proc TRAJ to start at the correct values. In

addition, the output from SAS shows the parameter estimates for linear, quadratic and cubic

functions along with the p-value for these estimates. Upon reviewing the data and graph for

group 2, it appeared that a cubic function was not the best shape to describe the graph and was

not a significant term within the model. The polynomial function with the most significant term

was the linear function and therefore group 2 was changed to a linear function within the SAS

code (see **Table 6** and SAS code).

Table 6: Parameter estimates for group 2 within the 6-group trajectory model

Group	Parameter	Estimate	Standard Error	T for Ho:Parameter =0	Prob >T
2	Intercept	9.21041	0.78743	11.697	0.00
	Linear	-0.20870	0.21220	-0.983	0.33*
	Quadratic	0.00933	0.01669	0.559	0.58
	Cubic	-0.00007	0.00038	-0.171	0.86

* Most significant term

```
Proc traj data=emily.WBC7traj out=OF outplot=OP outstat=OS;
Var W1-W14;
INDEP t1-t14; /* Time Variables */
MODEL CNORM; /* Censored Normal Model */
Start

    8.234277      0.330426 0.220357    -0.011015
    10.616873    -0.450828   /*0.037136     -0.000520    */
    21.212899    -1.852936       0.158936      -0.003631
    17.554821     3.764528     -0.476706      0.018936
    36.025168     0.062100   -0.530708      0.030677
    65.754552    -5.742047       0.605093      -0.011588
    -501.134540   273.102744      40.106874     -2.787700
    0.154716     -0.112028       -3.378010      0.010999
    0.034097     -5.934516      0.065027      0.037858
    -3.823199      0.197697     -0.141618     -4.323360
    0.050794      0.002176    5.727341     4.208319
    48.660504     32.043896      6.839595      7.375886
    0.871801 ;

MIN 0; /* Lower Censoring Point */
MAX 200; /* Upper Censoring Point */
NGROUPS 6; /* Fit 6 Groups */
ORDER 3 1 3 3 3 3; /* Cubic Trajectory for Each Group except group 2 which is
linear */
Obsmar icusurvival;
Dropout 2 2 2 2 2 2;
RUN;
```

I specified a linear (first order polynomial) for group 2, and kept the other groups at

cubic (third order polynomial). This resulted in a model that did not have a singular convergence

errors and the graphs are shown in **Figure 12**. The trajectories are similar to that obtained

initially for the 6-group model.
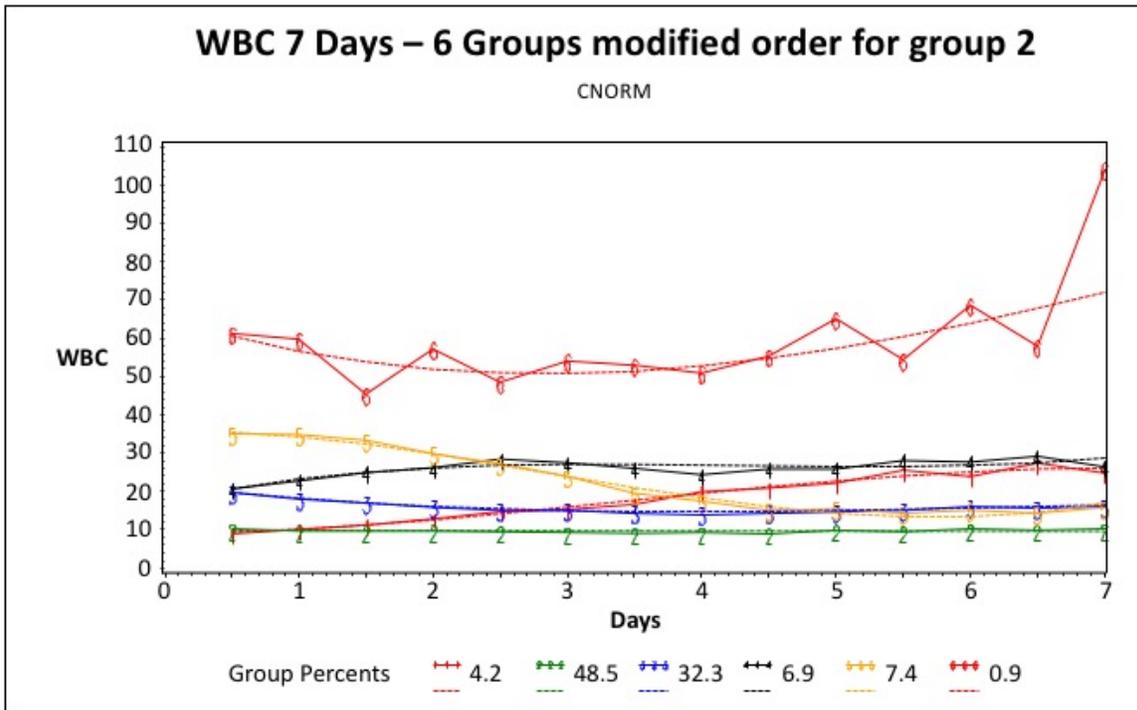
Figure 12: Graphical representation of the 6-group model from Proc TRAJ modified for group 2.
This plot is observing the trajectory of model using 6 groups, incorporating WBC data for 7 days
after admission to ICU

The 7-group trajectory model follows a similar pattern with group 1 showing a flat low trajectory (35%), group 2 showing a rising trajectory (8%), group 3 declining (35%), group 4 declining but starting at a higher value (14%), group 5 starting high and rising (3%), group 6 starting high and approaching normal by the end of the 7 days (5%) and the extreme WBC group (1%). This trajectory model is shown in **Figure 13**.
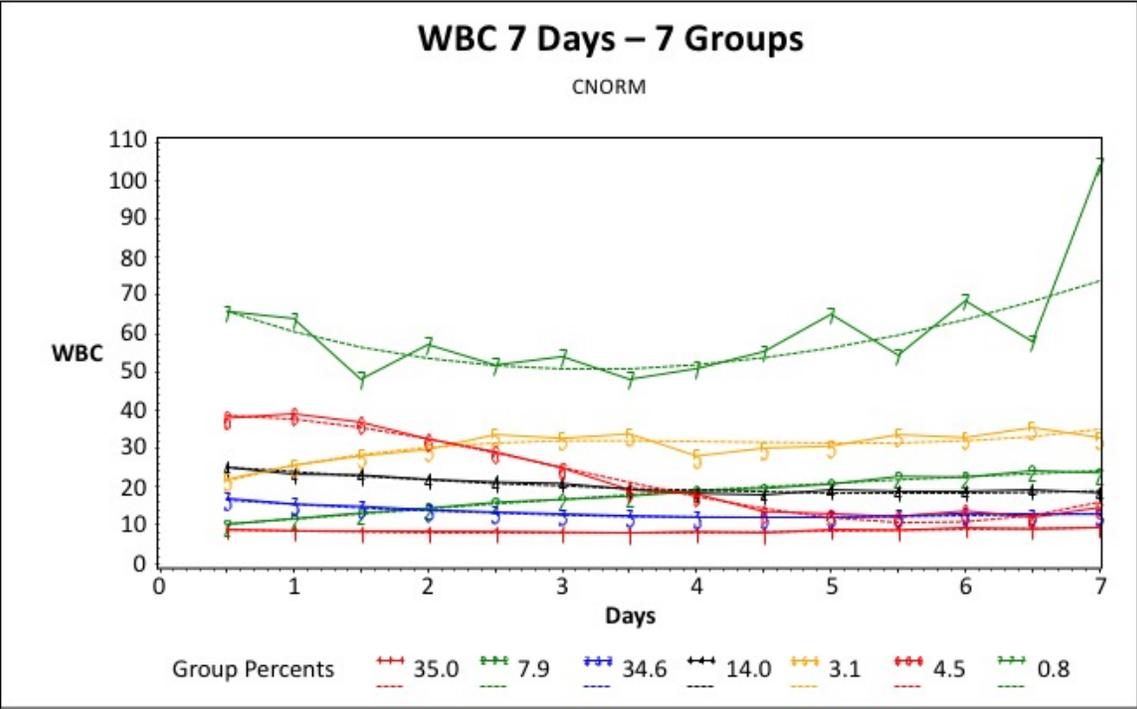

Figure 13: Graphical representation of the 7-group model from Proc TRAJ

This plot is observing the trajectory of model using 7 groups, incorporating WBC data for 7 days after admission to ICU.

Upon review with my thesis committee, the members determined that we should include 8, 9, and 10 group models to determine if the BIC continue to improve and to determine if a

group with a low total WBC count would emerge. The results of these are shown in **Figures 14-16**.
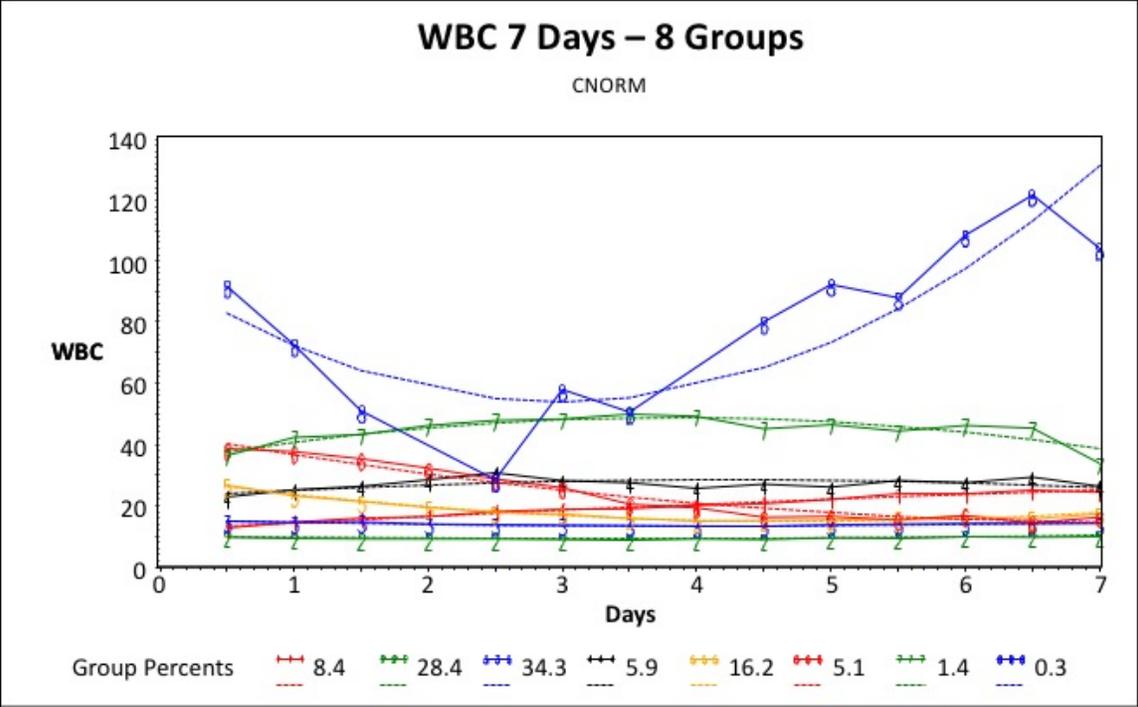
Figure 14: Graphical representation of the 8-group model from Proc TRAJ
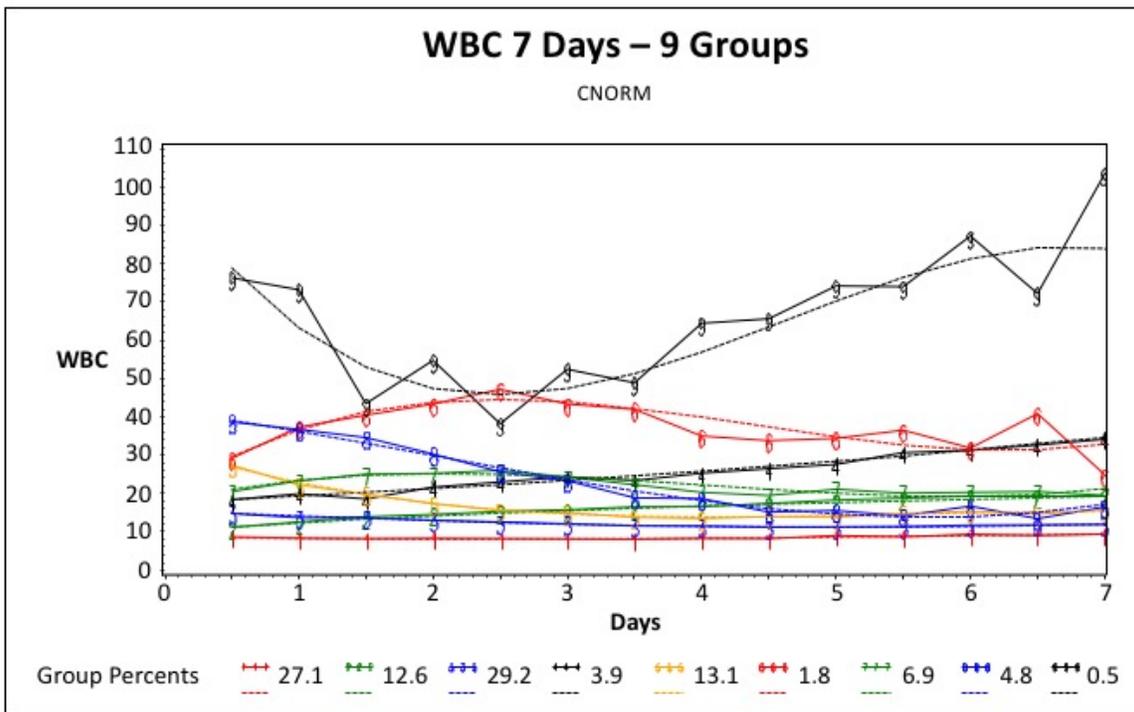
Figure 15: Graphical representation of the 9-group model from Proc TRAJ
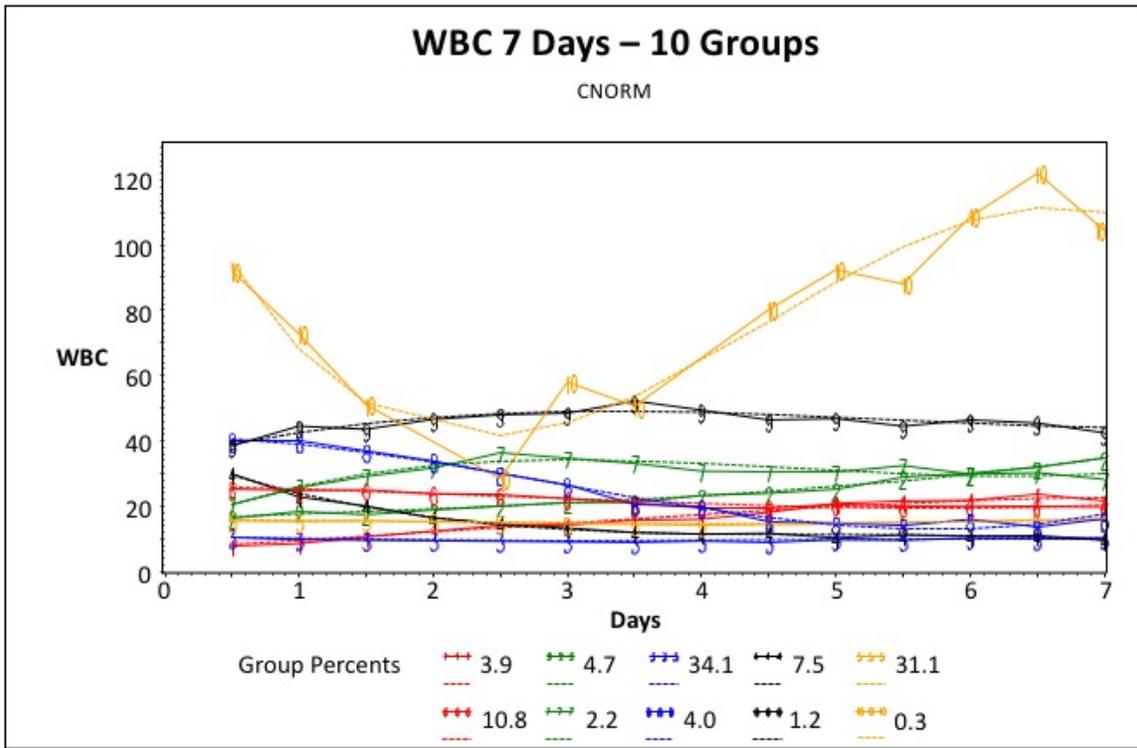
Figure 15: Graphical representation of the 10-group model from Proc TRAJ

After reviewing the graphs for clinical face-validity, the 5, 6, and 7 trajectory group models appeared to have the most face validity because these models differentiate patients into different trajectories that show a plausible WBC count response to treatment of infection. Some groups start high and normalize, others start normal or high and stay the same, while others have a WBC count that climbs despite treatment. The 8, 9, and 10 trajectory group models were felt to be too complicated to fully make sense of the patterns, had numerous groups with fewer than 5% of the population and failed to show a group with a low total WBC count. For these reasons, they were not included in further model building.

The next step in the process of finding the optimal number of trajectory groups is to assess the fit based on the BIC.

Assessing trajectory fit based on BIC output from Proc TRAJ

The BIC values for each trajectory model starting at 1 up to 7 are presented in **Table 7**. The BIC value with the smallest negative number (closest to zero) represents the best fit. The BIC values consistently become smaller as the number of groups increases. The BIC *observations* for 5 groups is -23648.3, for 6 groups is -23538.5, and for 7 groups is -23439.2. The logged Bayes factor, which is presented in **Table 8** for each comparison (6 vs 5, and 7 vs 6), shows that the more complicated model is favored. Based on BIC values alone, the model with 7 trajectory groups has the best fit.

Table 7: BIC values for total WBC trajectory groups 1 – 7

Number of Groups	BIC *observations*	BIC *subjects*
1	-26004.7	-25996.6
2	-24834.9	-24818.6
3	-24176.8	-24152.3
4	-23919.5	-23886.9
5	-23710.1	-23699.4
6	-23677.5	-23628.6
6 modified	-23674.6	-23627.8
7	-23499.2	-23442.2
8	-23238.4	-23181.34
9	-23254.5	-23181.2
10	-23135.9*	-23054.5*

*denotes smallest BIC

Table 8: Logged Bayes Factor for comparison between groups

Group Comparison	2*(ΔBIC)	Interpretation
6 vs 5	219.6	Very strong evidence for 6-group model
7 vs 6	198.6	Very strong evidence for 7-group model
8 vs 7	521.6	Very strong evidence for 8-group model
9 vs 8	-32.2	No evidence for 9-group model

10 vs 8	205	Very strong evidence for 10-group model
10 vs 9	237.2	Very strong evidence for 10-group model

Final model choice

The optimal model choice for Proc TRAJ is based on a combination of BIC results, parsimony and clinical judgement(33). The final model for WBC count trajectory in septic shock chosen for this study is the model with 7 trajectory groups. The reasons for choosing this model are:

1. The groups identified have clinical face validity and show meaningful and potentially expected trajectories for patients with septic shock

2. The BIC value is less negative than the preceding groups suggesting a better statistical fit compared to models with fewer groups. The BIC value increased in the 8 and 10 group models, however these models were complex and difficult to interpret so were not chosen as the final model.

3. The model with 7 groups illustrates a more representative distribution of the population among the 7 groups. For example, in the 6-group model group almost half of the population is contained within one group (group 2 – 49%)

4. Convergence error did not occur while modeling the 7-group trajectory model

Once the number of groups was finalized, the next step in model building was to vary the order of the polynomial within the 7 groups model to see if there is substantially better fit with a

different shape curve. I re-ran Proc TRAJ for a 7-group model, varying the order of polynomial for each group from 1 to 5. **Table 9** shows the BIC values for each degree of polynomial for the 7-group trajectory model. The results between the different degree of polynomial vary only minimally so I kept the final model at 3rd order polynomial. I felt that the 3rd order polynomial model has the most clinical face validity as this type of curve can model a WBC trajectory with 2 inflection points.

Table 9: BIC values for the 7-group trajectory model varying the degree of polynomial from 1st order to 5th order.

Degree of polynomial	BIC *observations*	BIC *subjects*
1	-23559.6	-23516.9
2	-23328.2	-23278.3
3	-23499.2	-23442.2
4	-23520.2	-23456.1
5	-23323.5	-23252.3

4.1.2 Description of the 7 trajectory groups for total WBC

As seen in **Figure 13**, I identified 7 groups with unique trajectories of the total white blood cell count in septic shock. Group 1 has a probability of group membership of 35%. This

group starts at a normal WBC count (approximately 10 x 10e9/L) and has a flat trajectory during the entire 7 days of study. Group 2 has a probability of group membership of 6% of the population. This group starts at a WBC count of approximately 10 x 10e9/L and within the first 1-2 days of observation, the WBC count starts to increase slowly and remains high for the duration of the first 7 days. Group 3 has a probability of group membership of 35% and starts out at a modestly increased WBC count (approximately 15 x 10e9/L) and gradually decreases over time. Group 4 has a probability of group membership of 14% and starts high (approximately 25 x 10e9/L) and decreases slowly over the 7-day period but always remains elevated. Group 5 has a probability of group membership 3% and starts out at a high WBC count (around 20 x 10e9/L). Over the first 2 days of ICU admission, the WBC count steadily rises and remains high during the entire 7-day period. Group 6 has a probability of group membership of 5% and starts with a very high WBC count (around 38 x 10e9/L) and rapidly falls over 3-5 days to a WBC value that is just above normal. Group 7 has a probability of group membership of 1% and has a significantly elevated WBC count that remains elevated throughout the first 7 days of ICU admission. These patterns seem, at face value, to be clinically meaningful. In some groups, the WBC count stays normal, in some in starts high and tends to resolve, but in some (groups 2 and 5) the WBC rises.

Table 10: Summary of WBC pattern for each group

Group Number	Summary of WBC trajectory
1	Normal, flat
2	Normal, rising
3	Moderate, gradual decline

4	High, gradual decline
5	High, rising
6	High, rapid decline
7	Significant elevation

4.1.3 Model diagnostics for the 7-group model

I assessed the model fit using 3 techniques as described in the *3.4.1 Model Diagnostics* section.

The first model diagnostic assesses at the ratio between the probability of group membership (πj) to the actual group membership. The probability of group membership measures the proportion of the population that belongs to a certain group. The ratio for each group should be close to 1. As seen in **Table 11**, the ratio between the predicted (probability of group membership) and the actual group membership for each group is very good, ranging from 0.82 to 1 with most groups having a ratio >0.95. This indicates that the model does a significantly good job of assigning to the correct group(33).

The second model diagnostic test is the average posterior probability (AvePP) for each group. The posterior probability is the probability that an *individual* with a specific pattern

belongs to a specific trajectory group and is calculated for each individual subject in Proc TRAJ. The AvePP is calculated by taking the average of the posterior probabilities for each trajectory group. Nagin suggests that each group should have an average posterior probability of greater than 0.7(33). The average posterior probability is shown in **Table 11** below. The average posterior probability for each group ranges from 0.82 (group 3) to 0.99 (group 7). All of the average posterior probabilities are greater than 0.7 which further suggests that the groups are correctly assigned.

The third diagnostic test is the Odds of Correct Classification (OCC) and is calculated by taking the ratio of the AvePP odds (AvePP/1-AvePP) to the πj odds ($\pi j/1-\pi j$). The higher the number the better the odds of being correctly classified are. Nagin suggests that the OCC should be >5 for each group. As seen in **Table 11**, all OCC values for groups 1-7 are greater than 5 which supports the assignments to the specific groups. The model diagnostics performed all supports a 7-group trajectory model.

Table 11: Model diagnostics for the 7-group trajectory model

Trajectory	πj (%)	Actual(%)	Ratio	πj odds	AvePP	AvePP odds	OCC
1	35	35.4	0.99	0.54	0.88	7.6	14.1
2	7.9	6.2	0.82	0.09	0.85	5.6	64.9
3	34.6	35.8	0.97	0.53	0.83	4.8	9.0
4	14	14.1	0.99	0.16	0.87	6.7	40.7
5	3.1	2.9	0.94	0.03	0.95	18.4	572.5
6	4.5	4.6	0.98	0.05	0.95	19.3	411.0

| 7 | 0.8 | 0.8 | 1 | 0.01 | 1.00 | 172412.8 | 2251348 |

4.2 Objective 2 - Assess associations between identified WBC trajectories and patient demographics, comorbidities, and pathogen characteristics.

4.2.1 Baseline characteristics of entire cohort

The final cohort for this study has 917 patients of whom 454 (50%) were males. The mean age of the entire cohort was 61 years (standard deviation (SD) 17 years) and the mean APACHE II score was 24 (SD 7). The median time to first antibiotics for the entire cohort was 2 hours (interquartile range (IQR) 0.03 to 6 hours) and 816 (89%) received appropriate antibiotics. 605 (72%) had a positive culture and 214 (23%) had bacteremia. The type of infection was gram positive in 254 (28%) of patients, gram negative in 258 (29%), fungal in 35 (4%) of patients and other in 40 (4%) of patients. Co-morbidities included diabetes mellitus as the most common in 366 (40%), followed by chronic obstructive pulmonary disease in 141 (15 %), and chronic kidney disease in 138 (15%). End-stage renal disease occurred in 114 (12%), and 6 patients (1%) had New York Heart Association class IV congestive heart failure. All baseline characteristics are presented in **Table 12**. There were significant differences between the different trajectory groups in baseline APACHE II score ($p<0.01$), time to first antibiotic ($p<0.01$), and baseline platelet count ($p<0.01$).

4.2.2 Characteristics of Group 1 (Normal, flat)

Group 1 comprised 325 patients (35% of total cohort) of whom 178 (55%) were male. The mean age of group 1 was 61 years (SD 16 years) and the mean APACHE II score was 23 (SD 7). The median time to first antibiotics for group 1 was 2.6 hours (IQR 0.4 to 6.3 hours) and

286 (88%) received appropriate antibiotics. There was a positive culture in 198 (67%) and 70

(22%) had bacteremia. The type of infection was gram positive in 76 (24%) of patients, gram

negative in 90 (28%), fungal in 12 (4%) of patients and 'other' in 14 (4%) of patients. Co-

morbidities were similar to the entire study cohort, with diabetes mellitus being the most

common in 124 (38%), followed by chronic obstructive pulmonary disease in 44 (14%), then

chronic kidney disease in 55 (17%). End-stage renal disease and dialysis-dependence at baseline

occurred in 45 (14%) had, and 5 patients (2%) had New York Heart Association class IV

congestive heart failure. All baseline characteristics for group 1 are presented in **Table 12**.

4.2.3 Characteristics of Group 2 (Normal, rising)

Group 2 comprised 57 patients (6% of total cohort) of whom 31 (54%) were male. The

mean age of group 2 was 57 years (SD 17 years) and the mean APACHE II score was 25 (SD 8).

The median time to first antibiotics for group 2 was 1.2 hours (IQR 0 to 5.7 hours) and 50 (88%)

received appropriate antibiotics. 45 (80.4%) had a positive culture and 13 (22.8%) had

bacteremia. The type of infection was gram positive in 25 (44.6%) of patients, gram negative in

13 (23.2%), fungal in 5 (8.9%) of patients and other in 1 (1.8%) of patients. Co-morbidities were

similar, with diabetes mellitus being the most common in 17 (29.8%), followed by chronic

obstructive pulmonary disease in 11 (19.3%), then chronic kidney disease in 7 (12.3%). 5

(8.8%) had end-stage renal disease and were on dialysis at baseline, and no patients had New

York Heart Association class IV congestive heart failure. All baseline characteristics for group 2

are presented in **Table 12**.

4.2.4 Characteristics of Group 3 (Moderate, gradual decline)

Group 3 comprised 329 patients (35.9% of total cohort) of whom 163 (49.5%) were male. The mean age of group 3 was 62.3 years (SD 16.6 years) and the mean APACHE II score was 23.2 (SD 6.9). The median time to first antibiotics for group 3 was 1.6 hours (IQR 0.02-5.6 hours) and 296 (90%) received appropriate antibiotics. There was a positive culture in 214 (71%) and 83 (25%) had bacteremia. The type of infection was gram positive in 93 (29%) patients, gram negative in 95 (29%), fungal in 10 (3%) of patients and other in 11 (3%) of patients. Diabetes mellitus was the most common co-morbidity in 129 (39%) patients, followed by chronic obstructive pulmonary disease in 54 (16%), then chronic kidney disease in 44 (13%). There were 40 (12%) patients with end-stage renal disease and who were on dialysis at baseline, and 1 patient had New York Heart Association class IV congestive heart failure. All baseline characteristics for group 3 are presented in **Table 12.**

4.2.5 Characteristics of Group 4 (High, gradual decline)

Group 4 comprised 130 patients (14% of total cohort) of whom 55 (42%) were male. The mean age of group 4 was 60 years (SD 16 years) and the mean APACHE II score was 25 (SD 7). The median time to first antibiotics for group 4 was 1.3 hours (IQR 0 to 4.2 hours) and 114 (88%) received appropriate antibiotics. There were 94 (80%) patients who had a positive culture and 27 (21%) had bacteremia. Gram positive infections were the most common in 41 patients (33%), then gram negative in 34 (27%), fungal in 5 (4%) of patients and other in 8 (7%) of patients. Diabetes mellitus was again the most common co-morbidity in 67 (51.5%), followed by chronic obstructive pulmonary disease in 24 (18.5%), then chronic kidney disease in 19

(14.6%). 15 (11.5%) had end-stage renal disease and were on dialysis at baseline, and no

patients had New York Heart Association class IV congestive heart failure. All baseline

characteristics for group 4 are presented in **Table 12.**

4.2.6 Characteristics of Group 5 (High, rising)

Group 5 comprised 27 patients (3% of total cohort) of whom 9 (33.3%) were male. The

mean age of group 5 was 58 years (SD 15 years) and the mean APACHE II score was 27 (SD 7).

The median time to first antibiotics for group 5 was 2.8 hours (IQR 0 to 6.4 hours) and 24 (89%)

received appropriate antibiotics. There were 15 (65%) patients who had a positive culture and 8

(30%) had bacteremia. Gram positive infections were the most common in 9 patients (33%),

then gram negative in 4 patients (15%), fungal in 1 (4%) patient and other or missing in the

remaining patients. Diabetes mellitus was present in 10 (37%), no patients had chronic

obstructive pulmonary disease, 4 patients had chronic kidney disease (15%). There was 1 patient

who had end-stage renal disease and was on dialysis at baseline, and no patients had New York

Heart Association class IV congestive heart failure. All baseline characteristics for group 5 are

presented in **Table 12**.

4.2.7 Characteristics of Group 6 (High, rapid decline)

Group 6 comprised 42 patients (5% of total cohort) of whom 17 (41%) were male. The

mean age of group 6 was 60 years (SD 18 years) and the mean APACHE II score was 26 (SD 8).

The median time to first antibiotics for group 6 was 1.4 hours (IQR 0 to 4 hours) and 41 (98%)

received appropriate antibiotics. There were 32 (84%) patients who had a positive culture and 12

(28.6%) had bacteremia. Gram negative infections were the most common type of infection in 20 patients (48%), then gram positive in 8 patients (19%), fungal in 1 (2%) patient and other or missing in the remaining patients. Diabetes mellitus was present in 18 (43%), 8 patients (19%) had chronic obstructive pulmonary disease, 8 patients had chronic kidney disease (19%). There were 7 patients (17%) who were dialysis dependent at baseline, and no patients had New York Heart Association class IV congestive heart failure. All baseline characteristics for group 6 are presented in **Table 12**.

4.2.8 Characteristics of Group 7 (Significantly elevated)

Group 7 was the smallest group and comprised 7 patients (0.8% of total cohort) of whom 1 was male. The mean age of group 7 was 53 years (SD 21 years) and the mean APACHE II score was 28 (SD 6). The median time to first antibiotics for group 7 was 16.4 hours (IQR 0 to 71.2 hours) and 5 patients (71%) received appropriate antibiotics. All patients had a positive culture and 1 had bacteremia. Gram negative infections were present in 2 (28.6%) of patients, gram positive in 2 patients (28.6%), fungal in 1 (14.3%) patient and 2 (28.6%) were classified as having other types of infections. There was 1 patient who had diabetes mellitus, no patients with chronic obstructive pulmonary disease, 1 patients with chronic kidney disease and 1 patient was dialysis dependent at baseline. There were no patients with New York Heart Association class IV congestive heart failure. All baseline characteristics for group 7 are presented in **Table 12**.

4.2.9 Key differences in baseline characteristics between groups

There were significant differences in sex among the groups identified. Males were less frequent in group 5 (high, rising), 6 (high, rapid decline), and 7 (significantly elevated) compared

with the other groups. Likewise, these groups also had higher APACHE II scores. An important distinction about group 7 (significantly elevated) is that the median time to antibiotics was 16.4 hours compared to 2 hours for the entire cohort. Similarly, groups 2 (normal, rising), 4 (high, gradual decline) and 6 (high, rapid decline) appeared to have a faster time to antibiotics compared to the rest of the groups. The platelet count was higher in groups 4 (high, gradual decline), 6 (high, rapid decline) and 7 (significantly elevated) compared to the other groups. The type of infection was also different between the groups, group 6 (high, rapid decline) seemed to have a higher rate of urinary tract infection compared to the other groups and the entire cohort as a whole. This may be of importance because the patients with urinary infection as the source of sepsis are more likely to have an improved survival. Group 2 (normal, rising) had a high number of gram positive infection (45%) compared to 28% for the entire cohort. Likewise, this may have an effect on survival of this group.

Table 12: Baseline characteristics of the entire cohort and each trajectory group

| | Entire Cohort | WBC Trajectory Groups | | | | | | | P-value (differences between groups) |
		1 Normal, flat	2 Normal, rising	3 Moderate, gradual decline	4 High, gradual decline	5 High, rising	6 High, rapid decline	7 Significantly elevated	
General Demographics	**N=917**	**N=325**	**N=57**	**N=329**	**N=130**	**N=27**	**N=42**	**N=7**	
Male, n, (%)	454 (49.5%)	178 (54.8%)	31 (54.4%)	163 (49.5%)	55 (42.3%)	9 (33.3%)	17 (40.5%)	1 (14.3%)	0.02*
Age (yr), mean (SD)	60.9 ± 16.5	60.7 ± 16.5	57.1 ± 17.1	62.3 ± 16.6	60.2 ± 15.5	58.4 ± 15.3	60.3 ± 17.8	53.1 ± 21.1	0.24
Duration of hospitalization before shock (median, IQR)	1 (0 - 2)	1 (0 - 3)	1 (0 - 2)	0.82 (0 - 2)	0.95 (0 - 2)	0.89 (0 - 2)	1 (0 - 4)	0 (0 - 3)	0.90
APACHE II score, mean (SD)	23.7 ± 7.2	22.7 ± 7	24.5 ± 8.1	23.2 ± 6.9	25.3 ± 7.2	26.7 ± 7.5	26 ± 7.7	27.7 ± 5.8	0.0002*
Time to 1st antibiotic (hrs), median, IQR	2 (0.03 - 6)	2.6 (0.4 - 6.3)	1.2 (0 - 5.7)	1.6 (0.02 - 5.6)	1.3 (0 - 4.2)	2.8 (0 - 6.4)	1.4 (0.08 - 4)	16.4 (0 - 71.2)	0.007*
Appropriate Antibiotic Use	816 (89%)	286 (88%)	50 (87.7%)	296 (90%)	114 (87.7%)	24 (88.9%)	41 (97.6%)	5 (71.4%)	0.38
Geographic Distribution									
Health Sciences Center	526 (57.4%)	192 (59.1%)	31 (54.4%)	186 (56.5%)	77 (59.2%)	13 (48.1%)	23 (54.8%)	4 (57.1%)	0.93
St. Boniface General Hospital	391 (42.6%)	133 (40.9%)	26 (45.6%)	143 (43.5%)	53 (40.8%)	14 (51.9%)	19 (45.2%)	3 (42.9%)	
Pre-existing medical Conditions, n (%)									
Chronic obstructive pulmonary disease	141 (15.4%)	44 (13.5%)	11 (19.3%)	54 (16.4%)	24 (18.5%)	0 (0%)	8 (19%)	0 (0%)	0.15
Diabetes mellitus	366 (39.9%)	124 (38.2%)	17 (29.8%)	129 (39.2%)	67 (51.5%)	10 (37%)	18 (42.9%)	1 (14.3%)	0.05*
Chronic Kidney Disease	138 (15%)	55 (16.9%)	7 (12.3%)	44 (13.4%)	19 (14.6%)	4 (14.8%)	8 (19%)	1 (14.3%)	0.87
Dialysis dependence	114 (12.4%)	45 (13.8%)	5 (8.8%)	40 (12.2%)	15 (11.5%)	1 (3.7%)	7 (16.7%)	1 (14.3%)	0.67
NYHA Class IV	6 (0.7%)	5 (1.5%)	0 (0%)	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.39
Physiologic and laboratory variables on admission [median (quartiles)]*									

Platelets (x10e9/L)	183 (118 - 259)	165 (106 - 224)	164 (107 - 245)	186.5 (128 - 266.5)	216 (159 - 313)	186.5 (87 - 312)	213 (122 - 339)	272 (154 - 311)	<.0001*
Serum creatinine (umol/L)	175 (98 - 329)	164 (90 - 338.5)	200 (117 - 262)	170 (101 - 311)	192 (98 - 351)	206 (113 - 294)	206 (105 - 314)	160 (94 - 356)	0.85
INR	1.4 (1.2 - 1.7)	1.4 (1.2 - 1.7)	1.4 (1.2 - 1.6)	1.4 (1.2 - 1.7)	1.4 (1.2 - 1.7)	1.4 (1.3 - 1.9)	1.4 (1.2 - 2)	1.8 (1.4 - 1.9)	0.31
Bilirubin (umol/L)	11.4 (7 - 23)	11 (7 - 22)	15.5 (8 - 24)	12 (6 - 24)	12 (7 - 32)	10 (7 - 23)	9 (7 - 17)	10 (5 - 41)	0.31
HCO$_3$ (meq/L)	19 (16 - 23)	19.5 (16 - 23)	20 (16 - 22)	19 (16 - 23)	18.8 (15.9 - 22)	19 (17 - 22)	18 (14 - 19.5)	20 (12 - 21)	0.17
Culture positive	605 (72.2%)	198 (66.9%)	45 (80.4%)	214 (71.3%)	94 (79.7%)	15 (65.2%)	32 (84.2%)	7 (100%)	0.02*
Bacteremia	214 (23.3%)	70 (21.5%)	13 (22.8%)	83 (25.2%)	27 (20.8%)	8 (29.6%)	12 (28.6%)	1 (14.3%)	0.77
Site of Infection, n (%)									
Catheter-related infection	16 (1.7%)	8 (2.5%)	0 (0%)	6 (1.8%)	1 (0.8%)	1 (3.7%)	0 (0%)	0 (0%)	0.65
Respiratory	324 (35.3%)	121 (37.2%)	22 (38.6%)	105 (31.9%)	52 (40%)	12 (44.4%)	9 (21.4%)	3 (42.9%)	0.21
Urinary Tract	96 (10.5%)	22 (6.8%)	1 (1.8%)	47 (14.3%)	11 (8.5%)	5 (18.5%)	10 (23.8%)	0 (0%)	0.0003*
Intra-abdominal	290 (31.6%)	102 (31.4%)	23 (40.4%)	111 (33.7%)	30 (23.1%)	5 (18.5%)	16 (38.1%)	3 (42.9%)	0.11
CNS	10 (1.1%)	1 (0.3%)	0 (0%)	3 (0.9%)	5 (3.8%)	1 (3.7%)	0 (0%)	0 (0%)	0.03*
Skin and soft tissue	124 (13.5%)	43 (13.2%)	8 (14%)	40 (12.2%)	24 (18.5%)	2 (7.4%)	6 (14.3%)	1 (14.3%)	0.65
Surgical site	8 (0.9%)	5 (1.5%)	1 (1.8%)	0 (0%)	2 (1.5%)	0 (0%)	0 (0%)	0 (0%)	0.38
Cardiac/Pericardial	3 (0.3%)	1 (0.3%)	0 (0%)	2 (0.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.02
Other	19 (2.1%)	9 (2.8%)	2 (3.5%)	3 (0.9%)	4 (3.1%)	1 (3.7%)	0 (0%)	0 (0%)	0.47
Type of Infection, n (%)									
Gram Positive Organism	254 (28.3%)	76 (23.8%)	25 (44.6%)	93 (28.7%)	41 (33.1%)	9 (33.3%)	8 (19%)	2 (28.6%)	0.03*
Gram Negative Organism	258 (28.7%)	90 (28.2%)	13 (23.2%)	95 (29.3%)	34 (27.4%)	4 (14.8%)	20 (47.6%)	2 (28.6%)	0.09
Fungal	35 (3.9%)	12 (3.8%)	5 (8.9%)	10 (3.1%)	5 (4%)	1 (3.7%)	1 (2.4%)	1 (14.3%)	0.35
Other	40 (4.4%)	14 (4.4%)	1 (1.8%)	11 (3.4%)	8 (6.5%)	1 (3.7%)	3 (7.1%)	2 (28.6%)	0.04*
New Organ Failures on Admission, n (%)**									
Renal failure	509 (55.5%)	171 (52.6%)	39 (68.4%)	173 (52.6%)	74 (56.9%)	19 (70.4%)	28 (66.7%)	5 (71.4%)	0.08

Hematologic failure	113 (12.3%)	49 (15.1%)	9 (15.8%)	32 (9.7%)	13 (10%)	5 (18.5%)	4 (9.5%)	1 (14.3%)	0.33
Hepatic failure	145 (15.8%)	43 (13.2%)	10 (17.5%)	54 (16.4%)	29 (22.3%)	5 (18.5%)	3 (7.1%)	1 (14.3%)	0.20
Respiratory failure	733 (79.9%)	268 (82.5%)	52 (91.2%)	253 (76.9%)	100 (76.9%)	22 (81.5%)	33 (78.6%)	5 (71.4%)	0.18
CNS failure	292 (31.8%)	111 (34.2%)	15 (26.3%)	100 (30.4%)	44 (33.8%)	10 (37%)	10 (23.8%)	2 (28.6%)	0.71
Coagulation failure	315 (34.4%)	106 (32.6%)	17 (29.8%)	106 (32.2%)	53 (40.8%)	11 (40.7%)	18 (42.9%)	4 (57.1%)	0.28
Metabolic failure	391 (42.6%)	122 (37.5%)	31 (54.4%)	137 (41.6%)	57 (43.8%)	14 (51.9%)	27 (64.3%)	3 (42.9%)	0.01*
Total number of day one organ failures (mean, SD)	3.7 ± 1.4	3.7 ± 1.4	4 ± 1.2	3.6 ± 1.5	3.8 ± 1.5	4.2 ± 1.5	3.9 ± 1.5	4 ± 1.9	0.12

*Denotes significant result

APACHE II = acute physiology and chronic health evaluation II; SD = standard deviation; IQR=interquatile range; yr=year; hr=hour; NYHA=New York Heart Association; HCO3=bicarbonate; CNS=central nervous system; INR=International Normalized Ratio. **Organ Failure definitions (Cardiovascular = systolic blood pressure <90mmHg or more than 40mmHg drop from normal OR MAP <65 mmHg for at least one hour despite adequate fluid resuscitation (2L saline or equivalent) OR the use of vasopressors; Renal = elevation of normal baseline creatinine to >1.5 x normal value; Respiratory = ventilation required; Hematologic = platelet count <80 x 10 e9/L; Metabolic = lactate >3mmol/L)

4.2.9 Results of univariable multinomial regression model to determine factors associated with trajectory group

I constructed a multinomial regression model to determine if baseline patient, treatment and illness characteristics were associated with WBC count trajectory group assignment in septic shock. I initially constructed a series of univariable regression models to determine their association with trajectory group. The results of these analysis are shown in **Table 13**. Variables that were tested include general demographics (age, sex, APACHE II score, time to first antibiotic, use of appropriate antibiotics, number of organ failures on day 1 of ICU admission), co-morbidities (COPD, diabetes, chronic kidney disease, dialysis dependence, NYHA class 4 heart failure), baseline platelet count and baseline creatinine, site of infection, type of infection (gram positive, gram negative, fungal or other). Variables were selected based on their clinical significance in septic shock and were selected based on input from a team of clinical experts in septic shock.

In a univariable model, baseline platelet count was associated with WBC count trajectory group. A higher platelet count was associated with a 2.3 (95% confidence interval (CI) 1.59 to 3.34) increase in odds of being in group 3 (moderate, gradual decline) compared to group 1 (normal, flat), a 3.36 (95% CI 2.15 to 5.24) increase in the odds of being in group 4 (high, gradual decline) compared to group 1, a 3.43 (95% CI 1.8 to 6.55) increase odds of being in group 6 (high, rapid decline), and a 1.16 (95% CI 1.02 to 1.31) increase in odds of being in group 7 (significantly elevated). Male sex (OR 2.42 (95% CI 1.06 to 5.55) and higher APACHE II score (OR 1.05 (95% CI 1.05 to 1.11) were both significantly associated with group 5 (high, rising). The results of all univariable models are presented in **Table 13**.

The associations of baseline characteristics and infection characteristics with WBC count trajectory are generally weak (i.e OR <3). For example, the association of sex and APACHE II score with group 5 have OR of 2.42 and 1.05 respectively. The clinical relevance of these weak associations is not known. As shown in **Table 13,** there are variables which have higher OR but which were not statistically significant. A pertinent example is the use of appropriate antibiotics, which is a critically important treatment of septic shock, has an OR of 5.59 for group 5 (high, rising) (95% CI 0.75 to 41.79). This means that *not* receiving appropriate antibiotics, that is administration of antibiotics with *in vitro* activity for the isolated pathogen or appropriate for the clinical syndrome in cases where no pathogen is isolated, has an increased odds of being in group 5 of almost 6-fold. While this is likely to be clinically significant, the results of the analysis were not statistically significant and raises the possibility of a type II error. The sample size in this cohort may not have been large enough to have the power to detect this difference with statistical significance.

Table 13: Factors associated with WBC trajectory on univariable multinomial regression model

Predictor	1 Normal, flat (ref)	2 Normal, rising	3 Moderate, gradual decline	4 High, gradual decline	5 High, rising	6 High, rapid decline	7 Significantly elevated
		OR (95% CI)					
General Demographics:							
Age		0.99 (0.97, 1)*	1.01 (1, 1.02)*	1 (0.99, 1.01)	0.99 (0.97, 1.02)	1 (0.98, 1.02)	0.97 (0.93, 1.02)
Female sex		1.02 (0.58, 1.79)	1.23 (0.91, 1.68)	1.65 (1.09, 2.49)	2.42 (1.06, 5.55)*	1.78 (0.93, 3.42)	7.27 (0.86, 61.03)
APACHE II score		1.03 (0.99, 1.07)	1 (0.98, 1.02)	1.02 (1, 1.05)	1.05 (1, 1.11)*	1.02 (0.98, 1.07)	1.04 (0.94, 1.15)
Number of organ failures on day 1		1.18 (0.98, 1.44)	0.96 (0.86, 1.07)	1.08 (0.94, 1.25)	1.27 (0.97, 1.65)	1.13 (0.9, 1.41)	1.17 (0.7, 1.94)
Time to 1st antibiotic (hrs)		1 (0.98, 1.02)	1 (0.99, 1.01)	0.99 (0.98, 1.01)	0.99 (0.96, 1.03)	0.95 (0.89, 1.02)	1.02 (1.01, 1.04)
Appropriate Antibiotic Use		0.97 (0.41, 2.3)	1.22 (0.75, 2)	0.97 (0.52, 1.81)	1.09 (0.31, 3.79)	5.59 (0.75, 41.79)	0.34 (0.06, 1.82)
Comorbidities:							
Chronic obstructive pulmonary disease		1.53 (0.74, 3.17)	1.25 (0.81, 1.93)	1.45 (0.84, 2.49)	N/a	1.5 (0.65, 3.46)	N/a
Diabetes mellitus		0.69 (0.37, 1.27)	1.05 (0.76, 1.43)	1.72 (1.14, 2.6)*	0.95 (0.42, 2.15)	1.22 (0.63, 2.33)	0.27 (0.03, 2.27)
Chronic kidney disease		0.69 (0.3, 1.6)	0.76 (0.49, 1.17)	0.84 (0.48, 1.48)	0.85 (0.28, 2.57)	1.16 (0.51, 2.63)	0.82 (0.1, 6.93)

Dialysis dependence		0.6 (0.23, 1.58)	0.86 (0.55, 1.36)	0.81 (0.44, 1.51)	0.24 (0.03, 1.81)	1.24 (0.52, 2.97)	1.04 (0.12, 8.82)
NYHA class IV		N/a	N/a	N/a	N/a	N/a	N/a
Baseline Lab Variables							
Platelet count (per 10 increase)		1.18 (0.59, 2.38)	2.3 (1.59, 3.34)*	3.36 (2.15, 5.24)*	2.3 (0.98, 5.4)	3.43 (1.8, 6.55)*	1.16 (1.02, 1.31)*
Creatinine		1.01 (0.98, 1.04)	0.99 (0.98, 1.01)	1 (0.98, 1.03)	0.97 (0.91, 1.03)	0.99 (0.94, 1.03)	0.99 (0.91, 1.08)
Presumed or documented site of infection:							
Culture positive		2.02 (1, 4.09)	1.23 (0.87, 1.74)	1.94 (1.16, 3.23)	0.93 (0.38, 2.26)	2.64 (1.07, 6.52)	N/a
Bacteremia		N/a	N/a	N/a	N/a	N/a	N/a
Catheter-related infection		N/a	N/a	N/a	N/a	N/a	N/a
Respiratory		N/a	N/a	N/a	N/a	N/a	N/a
Urinary Tract		N/a	N/a	N/a	N/a	N/a	N/a
Intra-abdominal		Ref	Ref	Ref	Ref	Ref	Ref
CNS		N/a	N/a	N/a	N/a	N/a	N/a
Skin and soft tissue		N/a	N/a	N/a	N/a	N/a	N/a
Surgical site		N/a	N/a	N/a	N/a	N/a	N/a
Cardiac/Pericardial		N/a	N/a	N/a	N/a	N/a	N/a
Other		0.56 (0.29, 1.1)	0.96 (0.7, 1.31)	0.67 (0.44, 1.01)	1.21 (0.55, 2.64)	0.62 (0.3, 1.28)	N/a
Culture positive		2.02 (1, 4.09)	1.23 (0.87, 1.74)	1.94 (1.16, 3.23)	0.93 (0.38, 2.26)	2.64 (1.07, 6.52)	N/a
Bacteremia		1.08 (0.55, 2.11)	1.23 (0.85, 1.77)	0.95 (0.58, 1.57)	1.53 (0.64, 3.65)	1.46 (0.71, 2.99)	0.61 (0.07, 5.13)
Type of infection:							
Gram Positive		Ref	Ref	Ref	Ref	Ref	Ref

Gram Negative		0.86 (0.44, 1.68)	1.11 (0.8, 1.56)	0.89 (0.58, 1.37)	0.57 (0.21, 1.55)	1.75 (0.92, 3.34)	N/a
Fungal		2.48 (0.96, 6.4)	0.88 (0.44, 1.76)	0.98 (0.41, 2.33)	1.06 (0.19, 5.84)	0.66 (0.12, 3.47)	N/a
Other		0.43 (0.08, 2.21)	0.83 (0.43, 1.61)	1.35 (0.64, 2.82)	0.91 (0.17, 4.96)	1.69 (0.56, 5.07)	N/a
Geographic Distribution:							
HSC		0.83 (0.47, 1.46)	0.9 (0.66, 1.23)	1.01 (0.67, 1.52)	0.64 (0.29, 1.41)	0.84 (0.44, 1.6)	0.92 (0.2, 4.19)
SBH		Ref	Ref	Ref	Ref	Ref	Ref

*denotes statistical significance

APACHE II = acute physiology and chronic health evaluation II; SD = standard deviation; IQR=interquatile range; yr=year; hr=hour; NYHA=New York Heart Association; HCO3=bicarbonate; CNS=central nervous system; INR=International Normalized Ratio; HSC=Health Sciences Centre; SBH= St Boniface Hospital

4.2.10 Results of multivariable multinomial regression model to determine factors associated with trajectory group

I constructed a multivariable multinomial regression model to assess the effects of multiple variables on trajectory group assignment. All variables that were felt to be clinically important were carried forward to the multivariable stage regardless of p-value in univariable testing. These variables included: age, sex, APACHE II score modified to remove the initial total WBC count, time to first antibiotic, use of appropriate antibiotics, total number of organ failures on day 1, bacteremia, and baseline platelet count.

The majority of variables were not significantly associated with trajectory group assignment. Increasing age (per year) was associated with a 1.01 increase in odds of being in group 3 (moderate, gradual decline) compared to group 1 (normal, flat) (95% CI 1 to 1.02). Females had an increase odds of being in group 4 (high, gradual decline) compared to group 1 (OR 1.87 (95%CI 1.18 to 2.97)). Increased time to first antibiotic was associated with group 7 (significantly elevated) (OR 1.03 (95% CI 1 to 1.05)). For every one-hour increase in time to first antibiotic, there was an increase in the odds of being in group 7 of 1.03. For every additional number of organ failures on day 1, there was a 1.38 increase in odds of being in group 6 (high, rapid decline) (OR 1.39 (95% CI 1.05 to 1.83)). The presence of bacteremia was associated with a 1.5-fold increase in odds of being in group 3 (moderate, gradual decline) (1.51 (95% CI 1.01 to 2.25). An increase in platelet count by 10 was associated with a 2.78 (95% CI 1.8 to 4.3) increase in odds of being in group 3 (moderate, gradual decline) compared to group 1, a 4.67 (95% CI 2.7 to 7.9) increase in odds of being in group 4 (high, gradual decline) compared to group 1, a 2.77 (95% CI 1.02 to 7.5) increase odds of being in group 5 (high, rising), and a

4.88 (95% CI 2.32 to 10.25) increase in odds of being in group 6 (high, rapid decline).   The

results of the multivariable model are presented in **Table 14**.

Table 14: Multivariable multinomial regression model to assess factors associated with trajectory group

Predictor	1 Normal, flat (ref)	2 Normal, rising	3 Moderate, gradual decline	4 High, gradual decline	5 High, rising	6 High, rapid decline	7 Significantly elevated
		OR (95% CI)					
age		0.99 (0.97, 1)	1.01 (1, 1.02)	1 (0.98, 1.01)	0.98 (0.95, 1.01)	1 (0.98, 1.02)	0.96 (0.91, 1.01)
male sex		0.89 (0.48, 1.65)	1.26 (0.9, 1.75)	1.87 (1.18, 2.97)	2.22 (0.89, 5.53)	1.93 (0.92, 4.06)	6.56 (0.69, 62.23)
APACHE II score (excl WBC)		1.02 (0.97, 1.07)	0.99 (0.96, 1.02)	1.02 (0.98, 1.05)	1.05 (0.98, 1.12)	1 (0.94, 1.06)	1.03 (0.91, 1.18)
Time to 1st antibiotic (hrs)		1 (0.98, 1.02)	1.01 (0.99, 1.02)	0.99 (0.97, 1.01)	0.99 (0.95, 1.03)	0.96 (0.9, 1.03)	1.03 (1, 1.05)
Appropriate Antibiotic Use		1.26 (0.42, 3.8)	1.73 (0.9, 3.31)	0.99 (0.43, 2.29)	0.85 (0.2, 3.67)	3.19 (0.38, 27.04)	0.98 (0.08, 11.83)
number of organ failures on day 1		1.21 (0.96, 1.53)	1.06 (0.93, 1.21)	1.17 (0.98, 1.4)	1.39 (1, 1.94)	1.39 (1.05, 1.83)	1.7 (0.88, 3.26)
bacteremia		0.98 (0.46, 2.11)	1.51 (1.01, 2.25)	1.32 (0.76, 2.31)	1.41 (0.51, 3.91)	1.85 (0.81, 4.23)	0.79 (0.08, 7.88)
PLT (per 10)		1.98 (0.92, 4.24)	2.78 (1.79, 4.3)	4.7 (2.77, 7.98)	2.77 (1.02, 7.5)	4.88 (2.32, 10.25)	3.93 (0.72, 21.51)
Hospital (HSC vs. SBH)		0.86 (0.63, 1.17)	0.99 (0.84, 1.18)	1.04 (0.82, 1.31)	0.82 (0.53, 1.29)	0.91 (0.63, 1.31)	1.13 (0.45, 2.8)

*denotes statistical significance
APACHE II=Acute Physiology and Chronic Health Evaluation II

4.3 Objective 3 - Assess the association between WBC trajectories and 30-day mortality using a Cox-proportional hazard model

4.3.1 Unadjusted mortality and clinical outcomes of the entire cohort and 7 trajectory groups

The unadjusted ICU mortality was 27% in this cohort of 917 patients with septic shock. The unadjusted 30-day mortality of the entire cohort was 26% (241/917) which is consistent with previous reports from the literature. The median ICU length of stay for the entire cohort was 8 days (IQR 4 to 14 days). Mechanical ventilation was required by 84% (771/917) and 20% required renal replacement therapy (186/917).

30-day, and ICU mortality

There was a significant difference in unadjusted ICU mortality (p= 0.003) and 30-day mortality (p=0.001) between the 7 trajectory groups. ICU mortality ranged from a low of 24.6% (80/325) in group 1 (normal, flat) to a high of 67% (18/27) in group 5. Likewise, 30-day mortality ranged from 23% (76/325) in group 1 to 63% (17/27) in group 5. The ICU and 30-day mortality rate among all groups are shown in **Table 15.** The groups with the highest mortality (groups 2 and 5) were those with rising WBC trajectories during ICU admission.

ICU length of stay, mechanical ventilation and renal replacement therapy

The median ICU length of stay was significantly different between the WBC count trajectory groups (p=0.02) and ranged from 7 to 10 days. There was no significant difference in the number of patients who required mechanical ventilation, however, there was a significant

difference in rates of renal replacement therapy (p<0.01). Renal replacement therapy was

implemented in approximately 15% of patients in groups 1 (normal, flat), 6 (high, rapid decline),

and 7 (significantly elevated) and 17% of patients in group 3 (moderate, gradual decline), 28% of

patients in group 4 (high, gradual decline), 35% of patients in group 2 (normal, rising), and 56%

of patients in group 5 (high, rising). These results are presented in **Table 15**.

Table 15: Unadjusted clinical outcomes of entire cohort and 7 trajectory groups

		WBC trajectory							
	Overall (n=917)	**1 Normal, flat (ref)** **N=325**	**2 Normal, rising** **N=57**	**3 Moderate, gradual decline** **N=329**	**4 High, gradual decline** **N=130**	**5 High, rising** **N=27**	**6 High, rapid decline** **N=42**	**7 Significantly elevated** **N=7**	**P-value**
30-day mortality, N (%)	241 (26.3%)	76 (23.4%)	18 (31.6%)	86 (26.1%)	30 (23.1%)	17 (63%)	12 (28.6%)	2 (28.6%)	0.0013
ICU mortality, N (%)	248 (27%)	80 (24.6%)	17 (29.8%)	86 (26.1%)	32 (24.6%)	18 (66.7%)	12 (28.6%)	3 (42.9%)	0.003
ICU length of stay, Median (IQR)	7.7 (4 - 13.6)	7 (4 - 13)	10 (7 - 16)	7 (4 - 13)	8 (4 - 14)	7.7 (4 - 21)	7 (4 - 11)	10 (4 - 28)	0.0206
Mechanical Ventilation, N (%)	771 (84.1%)	274 (84.3%)	54 (94.7%)	270 (82.1%)	107 (82.3%)	24 (88.9%)	35 (83.3%)	7 (100%)	0.2412
Renal replacement therapy, N (%)	186 (20.3%)	51 (15.7%)	20 (35.1%)	57 (17.3%)	36 (27.7%)	15 (55.6%)	6 (14.3%)	1 (14.3%)	<.0001

4.3.2 Cox-proportional hazard model assessing the effect of trajectory group on 30-day mortality

In a univariable Cox proportional hazard model, group 5 (high, rising) was associated with a significant increase in mortality at 30-days (hazard ratio (HR) 3.46 (95% CI 2.04 to 5.86), p<0.01). While the other trajectory groups were not significantly associated with 30-day mortality, groups 2 (normal, rising), 6 (high, rapid decline), and 7 (significantly elevated) were all associated numerical increases in the hazard of 30-day mortality. The results of the univariable Cox model are presented in **Table 16**. The groups with the highest numerical increase in the hazard of 30-day mortality were group 2 (HR 1.39, 95% CI 0.83 to 2.33, p=0.21) and group 5 (HR 3.46, 95% CI 2.04 to 5.86, p<0.01). These two groups had WBC count trajectories that were rising for the first 2-3 days of ICU admission despite treatment of septic shock.

Table 16: Univariable Cox-proportional hazard model evaluating the association of trajectory group on 30-day mortality

Trajectory group	Hazard Ratio (95% CI)	P-value
1 - Normal, flat	Ref	Ref
2 - Normal, rising	1.39 (0.83, 2.33)	0.21
3 - Moderate, gradual decline	1.12 (0.82, 1.53)	0.47
4 - High, gradual decline	0.97 (0.64, 1.48)	0.88
5 - High, rising	3.46 (2.04, 5.86)	<0.01

6 - High, rapid decline	1.29 (0.7, 2.37)	0.42
7 - Significant elevation	1.34 (0.33, 5.44)	0.69

After reviewing the results of the univariable Cox proportional hazard model, I then created a multivariable Cox proportional hazard model to assess the independent effect of WBC count trajectory on 30-day mortality while simultaneously accounting for other variables known or thought to be associated with mortality in septic shock. Included in this analysis were patient demographics (age, sex), illness variables (APACHE II score using the modified APACHE II - WBC variable, number of organ failures on day 1 of ICU admission), co-morbidities, pathogen variables (bacteremia, gram positive infection, gram negative infection, fungal infection, or other), co-intervention variables (time to first antibiotic, the provision of appropriate, or combination, antibiotics) and hospital. These variables were entered in the model regardless of p-value because they were all felt to be clinically relevant.

We found that group 5 (high, rising) was independently associated with an increased hazard of death at 30-days (HR 3.41, 95% CI 1.86 to 6.26, p<0.01). We also demonstrated that for every year increase in age, the risk of death increased by 1.2-fold (HR 1.2, 95%CI 1.07 to 1.32, p<0.01). Other factors significantly associated with 30-day mortality included APACHE II score modified for WBC (HR 1.08, 95% CI 1.06 to 1.1, p<0.01), NYHA class IV heart failure (HR 5.6, 95% CI 1.93 to 16.07), p<0.01), and *not* receiving appropriate antibiotics (HR 2.66, 95% CI 1.76 to 4.02, p<0.01). The results of the multivariable model are shown in **Table 17**.

Table 17: Results of multivariable Cox proportional hazard model

Trajectory Group	Hazard Ratio (95% CI)	P-value
Group 1 (reference)	Ref	Ref
Group 2	1.45 (0.83, 2.53)	0.19
Group 3	1.13 (0.81, 1.59)	0.46
Group 4	0.83 (0.5, 1.37)	0.47
Group 5	3.41 (1.86, 6.26)	<0.01
Group 6	1.42 (0.69, 2.91)	0.34
Group 7	1.46 (0.35, 6.12)	0.60
Age	1.2 (1.07, 1.32)	0.07
Female	1.01 (0.76, 1.34)	0.94
APACHE II - WBC	1.08 (1.06, 1.1)	<0.01
COPD	0.77 (0.51, 1.17)	0.22
Diabetes mellitus	0.83 (0.62, 1.12)	0.23
Dialysis dependent	1.25 (0.84, 1.87)	0.27
NYHA IV	5.57 (1.93, 16.07)	0.02
Bacteremia	0.9 (0.62, 1.32)	0.60
Gram positive infection	1.09 (0.72, 1.65)	0.69
Gram negative infection	1.13 (0.6, 2.1)	0.71
Fungal infection	1 (0.68, 1.48)	0.99
Other type of infection	2.03 (1.08, 3.82)	0.03
Delay in appropriate Abx (hours)	1.002 (0.99, 1.10)	0.53
No Appropriate Abx	2.66 (1.76, 4.02)	<0.01
HSC vs. SBH	0.93 (0.7, 1.23)	0.61

*denotes statistical significance
APACHE II = acute physiology and chronic health evaluation; COPD= chronic
obstructive pulmonary disease; NYHA=New York Heart Association;

Finally, a Kaplan-Meier survival curve was created to show the difference in

mortality among the 7 groups. As can be seen in **Figure 17**, group 5 (high, rising) has

the lowest survival compared to the other groups ($p<0.01$).
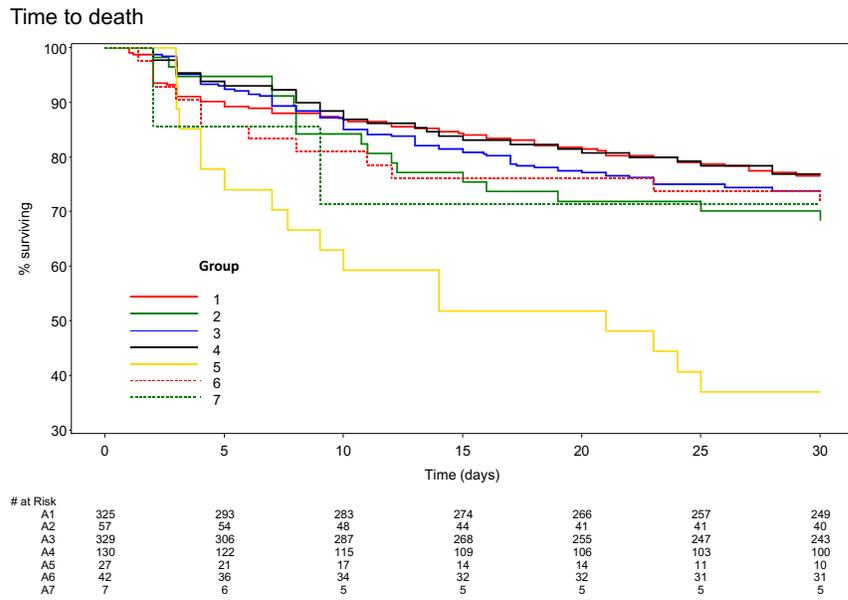
Figure 17: Kaplan Meier curve showing time to death to a maximum of 30 days.

Group 5 has the worst survival compared to other groups ($p<0.01$)

CHAPTER 5 - DISCUSSION

In this chapter, I will review the specific objectives of this study and review the results in the context of pre-existing literature in this area. A specific discussion regarding the implications of the results will be presented, followed by a discussion on the use of trajectory analysis in the medical literature. Strengths and limitations of this analysis will be presented and future applications of this methodology will be discussed.

5.1 Review of specific objectives

5.1.1 Use trajectory analysis to identify subgroups of patients with septic shock with differing patterns of WBC counts over time

In this retrospective cohort study of WBC count trajectory in septic shock, I was able to identify unique trajectories using the total WBC count. Trajectory analysis allowed me to demonstrate the presence of distinct and clinically relevant trajectories of the WBC in septic shock. Early on in model building groups started to emerge that showed unique patterns of WBC counts. Starting at 3 groups, there was a small but distinct group with a WBC pattern that was disproportionally elevated. This group was identified in each model including the final model with 7 groups. I also identified groups of patients with WBC counts that remained relatively stable over time, some that increased over time and others that declined.

Based on clinical face validity and the statistical assessment of model fit, the final model demonstrated 7 distinct trajectories of WBC counts during the first 7 days of ICU

admission for septic shock. Each of these groups have a unique pattern of the WBC

count, with different starting values, and various slopes depicting changes in WBC over

the next 7 days. The trajectories are again shown below in **Figure 18**.
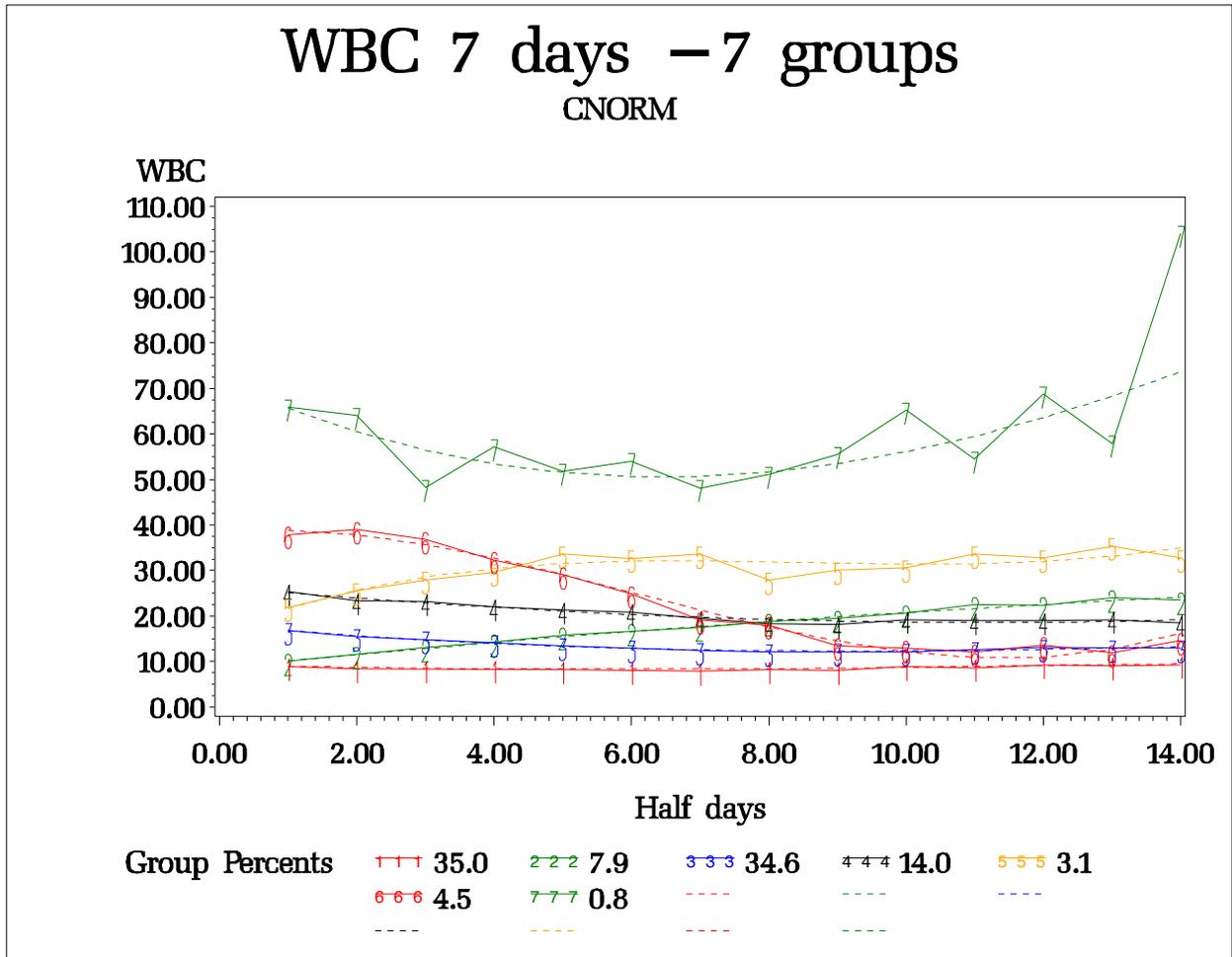
Figure 18. 7-group model for WBC trajectory in septic shock

Of particular interest, when I visually compare the pattern of each of the 7

trajectory groups to the mean WBC of the whole group (shown in **Figure 19**) and to the

trajectory plot for one group (shown in **Figure 20**), none of the 7 individual trajectories

follow the same pattern as the group as a whole.
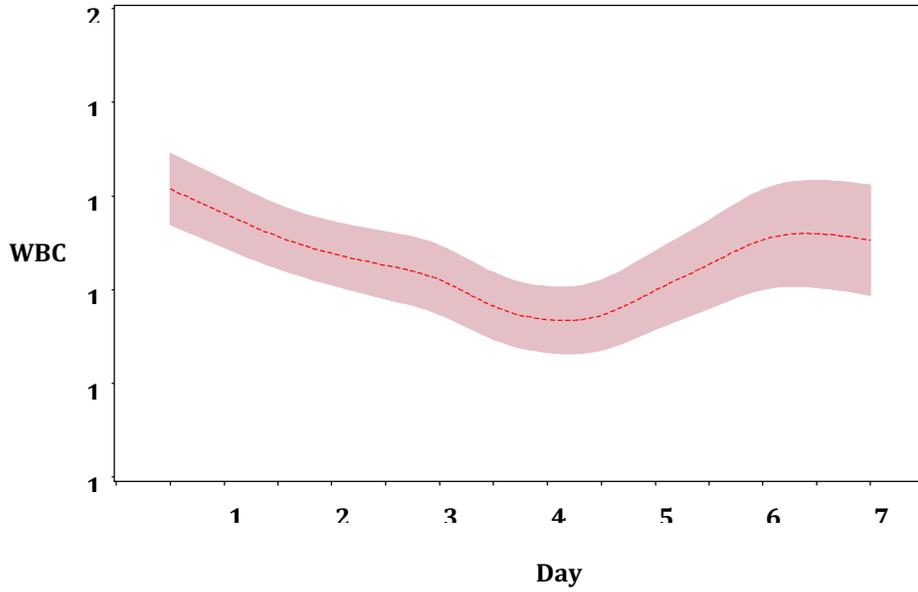
Figure 19: Plot of mean WBC over time with 95% confidence intervals
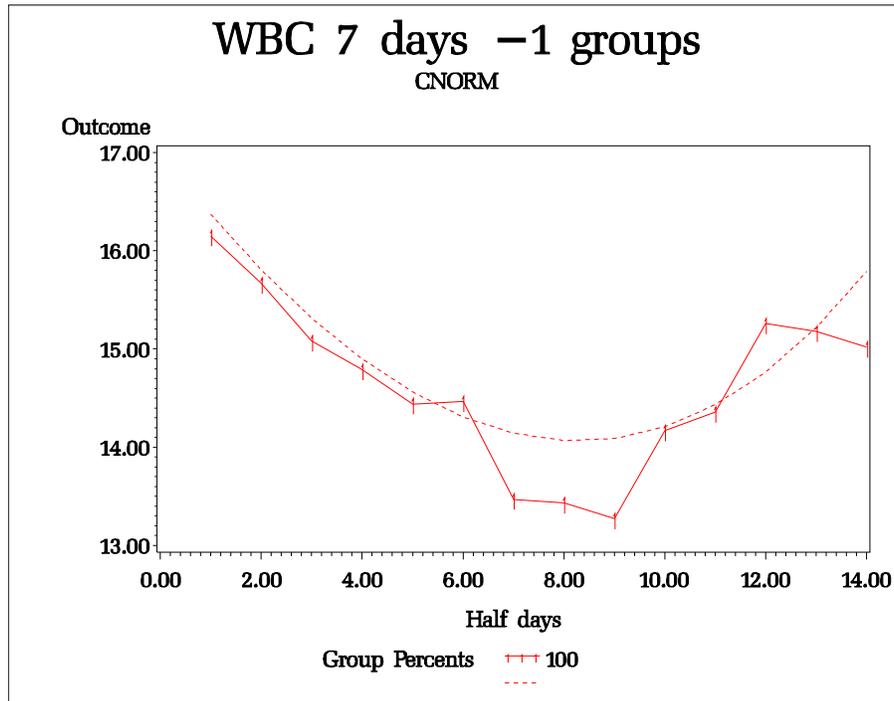
Figure 20: One group trajectory model for WBC in septic shock

The mean and 1-group trajectory model show a WBC that starts around 16, decreases slightly over the first couple days, and then increases again toward day 6 and 7. Interesting, the mean pattern (starts high and decreases over time) is what would be the "expected" pattern of the WBC response to infection, but in reality, when I perform the trajectory analyses, this specific pattern is not evident in any of the trajectory groups.

5.1.2 Identify characteristics of patients and pathogens associated with the different identified WBC trajectories

I performed a multinomial regression model to identify patient and illness specific characteristics that are associated with the different WBC trajectories. In this analysis, I identified characteristics associated with the various trajectories. Platelet count, and sex were associated with WBC count trajectory group in both univariable and multivariable analyses. It is possible that baseline platelet count is associated with WBC trajectory because they may both reflect bone marrow response to infection. A higher baseline platelet count was associated with higher odds of being in groups other than group 1 (normal, flat). Group 1 was the group with the lowest total WBC count at the time of admission, so it is likely that a bone marrow response of increasing the WBC count in response to infection, may also result in a higher baseline platelet count. It is well described that the platelet count in septic shock falls in response to severe infection(46), but the fall in the platelet count doesn't occur until a few days into the admission for septic shock(47). The underlying reason for the association of female sex with group 5 (high, rising) is less obvious. Perhaps the basis relates to underlying biologic differences in response to infection or other unmeasured variables. The majority of the variables

tested however, were not associated with trajectory suggesting that our knowledge of factors that influence the WBC count trajectory is incompletely understood.

5.1.3 Evaluate the association between the different WBC trajectories and 30-day mortality in patients with septic shock

In a multivariable Cox-proportional hazard model, we found that trajectory group 5 (high, rising) was independently associated with an increase in the risk of death at 30-days when accounting for age, sex, APACHE II score, number of organ failures on day 1 of ICU admission, co-morbidities, pathogen variables (bacteremia, gram positive infection, gram negative infection, fungal infection, or other) and co-intervention variables (time to first antibiotic, the provision of appropriate or combination antibiotics). There was a numerical increase in the hazard of 30-day mortality in group 2 which was not statistically significant. The trajectory of both of these groups showed a rising WBC count in the first 2-3 days of ICU admission, and raises the possibility that this pattern could provide a signal to treating clinicians that perhaps additional or alternative treatment may be required.

5.2 Why do unique WBC count trajectories exist in septic shock?

In a large cohort of patients with septic shock, by evaluating the trajectory of total WBC count, we identified distinct groups of patients that follow unique trajectories. We were unable to show that the WBC trajectory is easily predicted by baseline patient or illness characteristics such as age, sex, comorbidities, or infection characteristics. This raises the question, "what defines the uniqueness of these groups?"

There are many proposed sources of heterogeneity in sepsis that may account for the different trajectories observed. These can be broadly categorized into patient specific factors, illness factors and treatment factors(48). The patient or host specific factors may include baseline characteristics similar to what I assessed in this study, such as age, sex, and comorbidities, but also includes less tangible factors such as frailty, and specific differences in the host immune response to severe infection(48). It is possible that genetic or biologic differences exist in the patients who are assigned to specific WBC count trajectories. Perhaps these differences could be further assessed using gene array technology or by analysis of inflammatory biomarkers.

It is also plausible that infection specific factors may be different among the groups and these differences may influence a patients WBC count trajectory. The type of organism and virulence factors related to the organism type may, for example, be related to the WBC count trajectory. Although the organism type is available in the CATSS database, the absolute numbers for each specific organism in this study were too small to make any reliable inferences regarding the association with WBC count trajectory. Antibiotic susceptibility of the infectious organism is another factor that may be associated with WBC count trajectory but was not available in the dataset.

Treatment factors likely play a role in determining the WBC count trajectory of patients with septic shock. The duration of time between the onset of shock and initiation of appropriate treatment may play a role including the administration of prompt antibiotic therapy that is appropriate for the infection type, availability and timing of surgical

source control, amount of IV fluids that were administered, specific vasopressor used, or perhaps the availability and timing of other organ support measures. Many of these factors could plausibly have an important role in determining the trajectory of a particular group of patients.

The impact of these associations is unlikely to be fully appreciated in small datasets. Large databases, i.e. Big Data, that include clinical, laboratory, treatment data and possibly genetic information and inflammatory bio-markers will be required to comprehensively understand what factors contribute to WBC count trajectory group assignment.

5.3 What is the meaning of the different trajectories?

In this thesis, I have shown that group 5, which starts at a high WBC count of approximately 23 x 10e9/L and rises to a peak of about 30 x 10e9/L within the first couple days of the ICU admission, is independently associated with an increased risk of death at 30-days (HR 3.48, 95% CI 1.91 to 6.35, p<0.01). A similar rising trajectory is seen in group 2 but at a lower starting WBC count value. This group showed a similar trend toward increased risk of death that was not statistically significant (HR 1.46, 95% CI 0.84 to 2.54, p=0.18). It is possible that the *rising* trajectory can alert clinicians to patients who are on a trajectory that is associated with a poor outcome. With this knowledge, clinicians could then recognize a patient who could require closer monitoring, or who may require alternative treatment. Could additional source control be

required? Should the antibiotics be switched or intensified? Should these people be considered for clinical trials investigating additional therapies?

The groups with the lowest overall, unadjusted mortality were groups 1 (normal, flat) and 4 (high, gradual decline) (23.4% and 23.1% respectively). Both of these trajectories show a similar pattern as well, that is, relatively flat but slightly declining WBC count over the first few days of ICU admission. Unlike the groups with the rising trajectory, are these patients with stable WBC count trajectories likely to have better outcomes, and can we use this information to inform prognosis or clinical decision making? Should we design clinical trials to evaluate the utility of assessing WBC count trajectory before de-escalating or discontinuing antibiotics, or prior to transferring a patient to the ward?

It is also possible that the different trajectories may be unable to inform real-time patient-level decision-making but rather, yield phenotypic insights that further reflect host biologic differences in septic shock.

These questions are unlikely to be answered with small single institution studies and larger multi-center studies are required to validate these hypotheses.

5.4 How do the results of this thesis compare to results seen in literature?

There is a paucity of data pertaining to changes in the WBC count over time in patients with septic shock. As outlined in *Section 1.8 Complete Blood Count*, only few

small studies have described the WBC count in septic shock. One study reported that most patients had an initial leukocytosis for the first 48 hours of admission(16), characterized primarily by neutrophilia. In my thesis, when looking specifically at the WBC count trajectory over the first few days of ICU admission, many WBC trajectory groups have an initial leukocytosis. Interestingly, when I examined the *mean* WBC count over time, there is also an initial leukocytosis that normalized over four days. However, I similarly observed subtle differences that would be have been overlooked had I considered only the mean WBC count. These differences were evident early on in the course of the ICU admission. This contrast between the mean WBC profile, and the WBC count trajectories highlights one of the advantages of trajectory analysis in this type of investigation.

A study that evaluated neutrophil apoptosis in septic shock demonstrated that the WBC count at day 12 after admission was significantly higher in the non-survivors compared with survivors (35). This is consistent with what I observed. The groups with the lowest survival (groups 2 and 5) had both a rising WBC count trajectory and a WBC count at the end of the study period that was higher than the other groups.

Interestingly, although it has previously been shown that a low WBC count at onset of shock or ICU admission is associated with a higher hospital and ICU mortality (37), we did not identify a group with a low initial WBC count. This difference is plausibly explained by the exclusion of patients with hematologic malignancies, and patients who were receiving chemotherapy or immunosuppression. In addition, it is

possible that the patients with a low WBC count have been grouped into group 1 with the lowest WBC count. When examining group 1 a little more closely, the mean WBC count from this group was 8.6 x 10e9/L (SD 4.7) and the distribution of values within this group range from 0.2 to 22.9 x 10e9/L. The frequency of WBC count less than 4 x 10e9/L was 18.7%, 50.2% of the WBC counts were between 4 to 11 x 10e9/L, and 31% had values greater than 11 x10e9/L. In reviewing the individual data from group 1, there is almost 20% of this group who have an initial WBC count that is <4 x10e9/L.

5.4 How has trajectory analysis been used in medicine?

Trajectory analysis has been used extensively in the social sciences and has an increasing presence in medicine(49). In medicine, trajectory analysis has been used for a wide variety of purposes including to describe the pattern of thrombocytopenia in the course of hepatitis C virus treatment(50), and to define the unique patterns of transfusion and resuscitation in severe hemorrhage(51). Trajectory analysis has also been used to describe the association of viremia in human immunodeficiency viral infection on mortality(52), and to characterize sub-phenotypes of acute kidney injury based on the creatinine trajectory(53). A study of breast cancer patients showed that the hemoglobin trajectory within the first year of diagnosis was predictive of survival 10 years later(54). The purpose of many of these analyses was to describe differences within a population in order to characterize disease biology, describe health care utilization or identify patients who may have different outcomes or may benefit from additional therapies. Trajectory analysis is a well suited statistical procedure to identify and characterize these potential differences.

In medical research and clinical practice, our standard practice is to assess

prognosis based on a variety of baseline characteristics, for example, the APACHE II

score and SOFA scores as described above. More recently, we have recognized the

importance of temporal measurements in clinical assessment. We may still fail to

recognize differences in a group of patients but rather assess the entire group in

aggregate, for example, by using the mean platelet count in critical illness (55) and septic

shock(47). In the context of clinical medicine, trajectory analysis represents a relatively

novel method of assessing clinical data which will be further simplified by the use of

electronic medical records and large data repositories.

5.5 Strengths and Limitations

A strength of my thesis is the use of a novel method to analyze data using

trajectory analysis. Analyzing a large cohort of patients with septic shock, instead of

using the mean WBC count, I explored variability using trajectory analysis. I did not pre-

specify the groups, but allowed the data to guide the results and in the process identified

differences among the groups that are highly likely to be clinically relevant and suggest

future applications for this tool. Additional strengths include the use of the CATSS

database, which is, to my knowledge, the largest clinical database of septic shock

available. This database is derived from clinical data rather than relying solely on

administrative data which is an additional strength. I included all consecutive patients

who were admitted to the ICU at the included study sites. Further strengths include my

robust exclusion criteria whereby I excluded patients who had alternative explanations

for abnormalities in the WBC count (chemotherapy, other comorbidities known to affect the WBC count) which enabled me to assess the trajectory of the WBC count that is attributable to septic shock.

The analyses conducted are similarly limited by the novelty of the statistical methods. The process to arrive at the final trajectory model involved much trial and error and was, without question, an iterative process. I first used all data points available, but encountered statistical issues with excessive missing data. I then reduced the number of WBC counts used per day as the majority of patients would be getting only one CBC per day and therefore it didn't make clinical sense to use 4 measurements per day. Because of the high mortality associated with septic shock, I had to include a drop out function in the model to instruct Proc TRAJ which patients had missing data due to death. At the 6-group model, I encountered a convergence error and this required reviewing the output from SAS to specifying the starting values of the WBC count for each of the 6 group and altering the degree of the polynomial. A standard output from SAS is the parameter estimates and p-value for all level of polynomials, and therefore to improve the model fit, one is able to specify which level of polynomial function a particular group can follow based on the highest significant term.

An additional limitation is the retrospective nature of this study and therefore unmeasured confounders are potentially present. In addition, the database did not have access to certain information that might be useful such as antibiotic sensitivities. This was a descriptive study of 2 centers in a single city and the overall numbers are quite

small. Although I had access to extensive clinical data, much of it could not be analyzed because of small numbers in the various WBC trajectory groups. This analysis requires validation in a larger external dataset.

Finally, it is unknown if the variables I have chosen to analyze are the correct ones to explain the biologic variability in septic shock or to determine prognosis for patients in real time. Is the total WBC count the correct choice for this purpose? I chose the total WBC count for face-validity, familiarity among treating clinicians and generalizability to a non-hematologist knowledge-user. Perhaps the total neutrophil count or lymphocyte count may have been more instructive or provide additional information. It is also unknown if a single variable trajectory is the best model to take forward to understand the biologic differences among the groups; perhaps a multiple trajectory model including both platelet and WBC count plus other laboratory variables such as creatinine and/or bilirubin could result in more instructive and predictive trajectories.

5.6 Future Directions

To our knowledge, this is the first study, using trajectory analysis in septic shock and there are many questions that arise when considering the results produced. As previously mentioned, considerable thought went into choosing the primary variable to study. While the total WBC count was chosen for this study for its familiarity among clinicians and its direct role in sepsis and septic shock, it is possible that the trajectory of neutrophil counts and lymphocyte counts can also provide similar or supplementary information. The total WBC count is made up predominantly of neutrophils so it would be expected that a trajectory analysis based on neutrophil count in septic shock would follow a similar pattern to what I have shown in my analyses. Lymphocyte counts have been reported to be depressed in patients with septic shock, and as such, trajectory analysis of this parameter is likely to demonstrate something different to what was observed in the analysis of total WBC count. Both the analysis of the neutrophil and lymphocyte trajectories are planned as supplementary analysis after the completion of this study.

Similar to the abnormalities of the WBC count in septic shock, derangements in platelet count are common in patients with septic shock(47). While most studies evaluating the platelet count in septic shock have used the mean platelet count(55), including a large retrospective study at our institution(47), it is likely that the changes in platelet count over time in septic shock would be more accurately reflected using trajectory analysis. Our research group plans to build on the previous analysis of the time

course of the platelet count study in septic shock using trajectory methods to develop a more complete picture of thrombocytopenia in septic shock.

As previously mentioned (*Section 5.2 Why do unique WBC count trajectories exist in septic shock?),* it may be overly simplistic to think that the WBC trajectory alone is the ideal variable on which to model clinical heterogeneity in septic shock. Perhaps unique clinical phenotypes are more accurately depicted using a combination of variables such as the WBC count *and* the platelet count and/or other markers of severity of illness and comorbidities. The first step in assessing this will be to analyze the dual trajectory of WBC and platelet counts in septic shock.

In order to improve the validity and generalizability of this novel method of analysis, my results should be externally validated. A large, publically available US database of approximately 40,000 critical care patients (https://mimic.physionet.org/ Accessed March 13, 2018) might be suitable for this purpose.

Finally, the use of trajectory analysis can be expanded to other areas of clinical medicine. Trajectories can be potentially applied to characterize kidney dysfunction related to a number of nephrologic conditions, or predict outcomes based on response to treatment in malignancies where a biomarker can be followed (for example, prostate specific antigen for prostate cancer, or the monoclonal protein level in multiple myeloma). One example of the clinical utility is a study assessing the impact of hemoglobin trajectory in breast cancer(54). This study of almost 2000 patients with

breast cancer showed that the hemoglobin trajectory over the first 12 months following treatment of breast cancer was independently associated with survival 12 years later. Multiple possibilities for the application of trajectory analysis to characterize any disorder with repeated measurements or blood tests exists.

As medicine enters an era of "Big Data", large high-fidelity datasets are expected to be increasingly available to permit the application of trajectory analyses and other types of similar analyses. Big Data is defined as "an accumulation of data that is too large and complex for processing by traditional database management tools" (Merriam-Webster online dictionary). There are many other areas of information that can be brought into the Big Data realm in medicine (see **Figure 21**). By embracing the use of Big Data, we will be able to address many questions that we were unable to previously due to small samples and underpowered statistical capabilities. By evaluating 100,000 patients with septic shock instead of 1000 patients, we may have the power to detect what factors are associated with WBC trajectory and the ability to bring in additional data that is available outside of the realm of a retrospective study with a goal of understanding disease biology and host genotypes and other factors that may impact clinical outcome and response to treatment.

Figure 21: Sources of Big Data in Medicine.

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data? A primer for clinicians trained to think epidemiologically. Ann Am Thorac Soc

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CHAPTER 6 - CONCLUSIONS

In this retrospective cohort study of WBC trajectory in septic shock, we identified 7 unique and clinically relevant trajectories using the total WBC count. Baseline platelet count and male sex were associated with certain trajectory groups, however, due in part to the small absolute numbers within each trajectory group, I was unable to fully identify specific characteristics that could predict WBC trajectory. The rising WBC count trajectory (that was observed in group 5 and, to a lesser extent, group 2) was associated with an increase hazard of death in septic shock independent of powerful predictors of survival including antibiotic treatment and APACHE score.

The identification of distinct trajectories can be applied to further the understanding of disease biology in septic shock, and be applied to individualizing prognosis for a particular patient at a particular time and can perhaps, be applied to identifying individuals who may require additional therapies. In order to assess this question further, a large dataset with high fidelity clinical data will be required.

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