USING LATENT CLASS MIXTURE MODELS TO DEFINE SEPSIS ENDOTYPES

by

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ABSTRACT

Severe sepsis is associated with high mortality and is a common problem in the United States. Recently, studies have shown that efforts focused on lowering cytokine levels improve survival. The aim of this work is to define sepsis endotypes using longitudinal cytokine measurements.

Sepsis endotypes were defined using latent class mixture models. Latent class mixture models were modeled using a natural log transformation of the actual time measurements. The outcome was the natural log of the cytokine value. No other covariates were modeled and a parameterized link function using a basis of I-splines was chosen over a linear transformation to increase flexibility in the latent class trajectories. The number of latent classes were determined by a combination of the lowest BIC and clinical significance.

After creating models for a variety of subsets derived from the source population, it was determined that mortality within a particular trajectory class is not only dependent upon the baseline cytokine value, but also dependent upon the rate of decent after baseline. A class with high baseline cytokine values that decrease quickly has lower mortality rates than classes who do not decline quickly. It was also determined that those who have increasing cytokine values from baseline to 6 hours have worse outcomes than those who decrease in the same time frame.

Public Health Significance: Given the public health significance of sepsis, understanding prognosis is extremely important. Previously, having a high IL6 measurement implied a poor prognosis. Our results show that many factors play into the determination of prognosis and patients can be treated accordingly.

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PREFACE

I would like to thank my advisor, Dr. Joyce Chang, as well as Dr. Gilles Clermont and Dr. Ada Youk for their guidance and support with this project.

I would also like to thanks Dr. Jonathon Yabes and Dr. Francis Pike for their support and guidance throughout my graduate career. Lastly, I would like to thank my family for the continued support throughout my educational career.

1.0 INTRODUCTION

Severe sepsis is a common problem that is associated with a high mortality rate. In 1990, it was estimated that there were 450,000 cases of sepsis per year in the United States (Angus et al., 2001). In 2003, Hotchkiss et al. discussed circulating TNF levels and the relationship of high levels with mortality. The merits of lowering cytokine levels in septic patients has been shown in a recent study in which a subgroup of septic patients had improved survival when therapy was directed against TNF (Hotchkiss & Karl, 2003). An analysis of anti-inflammatory agents in septic patients also showed that a small subgroup, approximately 10%, benefited from high doses of these anti-inflammatory agents. (Hotchkiss & Karl, 2003). Many studies focusing on lowering cytokines levels at this time do not use longitudinal data but recently, the idea of latent canonical trajectory classes has been proposed. Defining trajectory classes allows septic patients to be placed into a specific endotype allowing for a more accurate understanding of prognosis. Endotyping involves placing patients into a subtype of the condition being studied and could be defined by a pathobiological mechanism or a treatment response. Endotyping helps to better assess risk and mortality.

The idea of endotyping with longitudinal cytokine measurements has been discussed by Kellum et al. in 2008. They describe the systemic cytokine response to pneumonia and determine if specific patterns are associated with severe sepsis and death (Kellum, 2008). It was determined that unbalanced, or high/low, cytokine patterns were unusual (4.6%) and not associated with

decreased survival. The highest risk of death occurred with combined high levels of the pro-inflammatory IL-6 and anti-inflammatory IL-10 cytokines (Kellum, 2008). This analysis used longitudinal daily cytokine measurements over the first week and modeled the cytokine trajectories as cubic polynomials over time. This analysis can be improved upon by using the Protocol-Based Care for Early Septic Shock (ProCESS) data. The ProCESS data was collected from a randomized trial at 31 hospitals in the United States.

The ProCESS data measures cytokines IL6 and Il10 at baseline (0 hours), 6 hours, 24 hours and 72 hours. This allows trajectories to have a better representation of the critical first day post enrollment. The ProCESS data set is more complete with data on patients that have increasing trajectories and is not limited to the systemic cytokine response to pneumonia. The main aim of this paper is to define endotypes within septic patients for a more accurate understanding of prognosis. To address this aim, latent classes were defined using latent class mixture models. Specifically, we looked for trajectory classes in all patients as well as those whose cytokine levels increased from baseline to 6 hours and in those whose cytokine levels decreased from baseline to 6 hours.

2.0 METHODS

2.1 STUDY POPULATION

The ProCESS study enrolled subjects at 31 hospitals in the United States. Subjects were recruited from the emergency department if sepsis was suspected according to the treating physician, they were at least 18 years of age, met two or more criteria for systemic inflammatory response syndrome and who had refractory hypotension or a serum lactate level of 4 mmol per liter or higher. Patients were excluded if a primary diagnosis of acute cerebral vascular event, acute coronary syndrome, acute pulmonary edema, status asthmaticus, major cardiac arrhythmia, active gastrointestinal hemorrhage, seizure, drug overdose, burn or trauma; a requirement for immediate surgery; a known CD4 count <50/mm2; an advance directive that would restrict protocol implementation; a contraindication to central venous catheterization; a high likelihood of refusing blood transfusion; a treating physician who deemed resuscitation to be futile; on-going participation in another interventional study; known pregnancy, or; been transferred from another hospital (Process Investigators, 2014). Patients were randomized to one of three treatment groups: protocol-based early goal directed therapy (EGDT), protocol-based standard therapy, or usual care in a 1:1:1 ratio. Based on the results of the original study, the three interventions were combined into one intervention group (Process Investigators, 2014).

Two cohorts of patients were used for the analysis. The complete measurement cohort was composed of patients who had IL6 and IL10 measurement at 0 hours, 6 hours and 24 hours at the minimum. Note that individuals with only the first three out of four time points were considered in the complete measurement set even though they are not a true complete measurement set.

Including these individuals allowed for a larger subset of the data. This subgroup may present a survivor bias, so an additional cohort was considered called the whole cohort. This group consisted of patients with at least one IL6 and at least one IL10 measurement.

The whole cohort and the complete measurement cohort were further divided into 2 groups: those who had an increasing cytokine measurement from hour 0 to hour 6 and those with a decreasing cytokine measurement from hour 0 to hour 6. These were referred to as the increasing/ decreasing subsets. Individuals whose cytokine measures remain constant from hour 0 to 6 are not included in these analyses.

All patients or their legal representatives provided an informed written consent. The institutional review board at the University of Pittsburgh and at all other participating site approved the study protocol.

2.2 MEASUREMENTS

The primary outcomes of the study were values for the cytokines IL6 and IL10. These cytokines were measured at 0 hours, represented by the entrance to the study, 6 hours, 24 hours and 72 hours. Mortality at 60 days for any reason was also measured. Demographic variables included sex, age and race (black, white or other). Baseline measurements reflecting status of health include Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score, number septic by definition, systolic blood pressure (SPB), diastolic blood pressure (DBP), heart rate, temperature, respiratory rate, lactate, white blood cell count and binary variables use of mechanical ventilation, pre-randomization use of vasopressor and

randomization to 6 hour vasopressor use. Time was measured as either the actual time (0, 6, 24 and 72 hours) or ln (hours+1) to determine the best fit for the data.

2.3 STATISTICAL METHODS LITERATURE REVIEW

In order to better understand the latent class mixture model, a brief description follows. A standard linear mixed model assumes that everyone in the population is homogeneous and assumes that everyone in the population is described by a unique trajectory. In contrast, latent class mixture models assume that the population is heterogeneous and comprised of G mean profiles of trajectories, or classes, where an individual can belong to only one latent class. Using this model, it is important to define a structural model for the latent process, a measurement model that links the latent process to the scale of the observation and define probability (Proust-Lima, Philipps, & Liquet, n.d.). Latent variables are variables that cannot be directly observed. Rather, they are inferred from other observed variables. The structural model models the latent, or unobserved, process and the measurement model converts the unobserved latent value into our outcome of interest, natural log of the cytokine value.

In the structural model, the latent process is defined in continuous time according to the standard linear mixed model below. In the structural model, fixed and random covariates can take on any polynomial shape. Also note that the model does not include measurement errors (Proust-Lima et al., n.d.).

$$\Lambda_i(t)|_{c_i=g} = X_{1i}(t)^T\beta + X_{2i}(t)^T\nu_g + \mathrm{Z}_i(\mathrm{t})^T\alpha_{ig}, \quad \forall t \in \mathbb{R}$$

where

$\Lambda_i(t)$ is the latent process of patient *i* where i = 1, 2, …, m

m is the number of patients

c_i is a latent random variable that equals g if patient *i* belongs to latent class g where g =1, 2, … G

G is the total number of classes

$X_{1i}(t)$ is a vectors of covariates for patient *i* associated with fixed effects that are common over latent classes

β is a vector of fixed effects that are common over latent classes

$X_{2i}(t)$ is a vector of covariates for patient i associated with fixed effects that differ by latent class

ν_g is a vector of fixed effects that differ depending on which latent class, g, a patient belongs

$\mathrm{Z}_i(\mathrm{t})$ is a vector of covariates for patient *i* associated with individual random effects

α_{ig} is a vector of individual random effects $\alpha_i|_{c_i=g}$ whose distributions are class specific

$\alpha_{ig} \sim N(0, \omega_g^2 B)$ where B is an unspecified variance covariance matrix and ω_g is a proportional coefficient that allows for class-specific variance-covariance matrices of the random-effects (In this paper, ω_g= 1 for all classes implying the variance-covariance matrix is common over latent classes)

t is time

The measurement model is defined between the latent processes $\Lambda_i(t)$ and the observed value Y_{ij} for individual *i* at time *j* shown below (Proust-Lima et al., n.d.). The measurement model that will be used incorporates the use of splines instead of using a linear model or modeling with polynomial functions.

$$Y_{ij} = H(\Lambda_i(t_{ij}) + \varepsilon_{ij};\ \eta)$$

Y_{ij} is the observed value for patient *i* at time *j* where *i* = 1, 2, …, m

m is the number of patients

H is a parameterized link function with parameters η and is defined by a basis of quadratic I-splines

$\Lambda_i(t_{ij})$ is the latent process of patient *i* at time *j*

ε_{ij} are the independent normal measurement errors with mean 0 and variance σ_ε^2

The parameterized link function is a basis of quadratic I-splines defined below. Splines extend the advantages of polynomials by allowing greater flexibility and are basically piecewise polynomials with specified continuity constraints. Continuity and number of parameters depend on a knot sequence. If the spline were to be defined on an interval [L, U], the knots divide the interval into a number of subintervals. A given knot sequence is associated with a suitable set of

basis splines that can be combined linearly to produce any other spline associated with this knot sequence. This set of basis functions is then modified to provide a useful basis for monotone splines, also known as I-splines. The I-spline is referred to as quadratic when the piecewise polynomials are quadratic in shape. A 4 knot spline will be used in all models to remain consistent (Ramsay, 1988).

$$H^{-1}(Y_{ij};\, \eta) = \eta_0 + \sum_{l=1}^{5} \eta_l^2\, \beta_l^I(Y_{ij})$$

H^{-1} is the inverse of the parametrized link function

Y_{ij} is the observed value for individual *i* at time *j* where *i* = 1, 2, …, m

m is the number of patients

η_i are the parameters of the link function, *i* = 0, 1, 2, 3, 4, 5

$(\beta_1^I, \beta_2^I, \beta_3^I, \beta_4^I, \beta_5^I)$ is the basis of I-splines

In the above parameterized link function, note that H^{-1} is a monotonic increasing continuous function. The parameters in the summation are squared in order to constrain the parameters to be positive.

The probability of belonging to any one specific latent class is defined using a multinomial logistic regression and is shown below. When no covariates predict latent class membership, the model reduces to a class specific probability (Proust-Lima et al., n.d.).

$$\pi_{ig} = P(c_i = g|X_{ci}) = \frac{e^{\zeta_{og} + {X_{ci}}^T \zeta_{1g}}}{\sum_{l=1}^{G} e^{\zeta_{ol} + {X_{ci}}^T \zeta_{1l}}},$$

where

π_{ig} is the probability that patient *i* belongs to class g where i = 1, 2, …, m and g = 1, 2, …, G

m is the number of patients

G is the number of classes

c_i is a latent random variable that equals g if subject *i* belongs to latent class g

X_{ci} are covariates in the multinomial logistic regression

ζ_{og} is the intercept to class g

ζ_{1g} are the class-specific parameters associated with the covariates X_{ci}

Bayesian Information Criterion (BIC) is a method that is commonly used to determine the number of latent classes in latent class mixture models. The model with the lowest BIC has the optimal number of classes. The formula for BIC is shown below.

$$BIC = -2L(\theta_G) + p_G \ln(N)$$

where

G is the number of latent classes

$L(\theta_G)$ is the log-likelihood of the model

p_G is the number of estimated parameters

N is the number of subjects

The BIC is preferred to the more well-known Akaike Information Criteria (AIC) as is favors more parsimonious models than AIC by penalizing for model complexity with ln(N) as compared to 2 in the AIC formula (Commenges & Jacqmin-Gadda, 2016).

2.4 STATISTICAL ANALYSIS

Statistical analyses will be performed using SAS version 9.4 and the R version 3.2.3 package LCMM. Statistical significance will be set at 0.05. All cytokine measurements will be analyzed in the natural logarithmic scale. If a cytokine has a value of 0 before the natural log transformation, its value will be changed to 1 in order to avoid undefined values post natural log transformation.

In order to make statistical comparisons between the whole and complete measurement sub groups, baseline analysis will be considered in the whole cohort, the complete measurement cohort and on the group of individuals who are in the whole cohort but not the complete measurement cohort, called the incomplete measurement cohort. This group is titled incomplete measurement cohort due to their lack of a complete measurement set.

Kaplan-Meier curves will be created for each of the two groups as well as in the incomplete measurement group to investigate any differences in mortality. Day of death will be used as the event time and time will be capped at 60 days as the administrative censoring day. Individuals will be censored at time of discharge if they were discharged from the hospital and a follow up was not completed.

In order to create trajectories for IL6 and IL10, latent class mixture models will be created using the R program LCMM. Latent class mixture models are preferred over other types of trajectory analyses (e.g., latent group-based trajectory models) because random deviations from the mean trajectory are allowed for people belonging to the same latent trajectory group.

In the structural model piece of the latent class mixture models, candidates for fixed covariates over classes will include time squared, race, sex and age. The class specific fixed covariates and the random covariates will include time squared only. Time is modeled as time squared for these covariates due to previous work that showed that the data is not linear in regard to time and quadratic shapes better reflect the data. Time in these models will also be investigated as either actual time or ln (hours+1). It is suspected that using ln (hours+1) allows for better modeling of the polynomial shapes of time as it is more evenly spaced than the original time measurement. The variance-covariance matrix for the random effects will be diagonal. This indicates that the variance is equal between two time points that are the same but the covariance between two differing time points is 0. The parameterized link function will use a basis of quadratic I-splines with 4 knots.

The number of latent classes will be determined by finding the lowest BIC. In order to maintain clinical significance, the number of groups may be decreased to avoid classes with a small percent of the total population. Groups will be preferred to have at least 8 percent of the total population so that classes are large enough to have clinical significance. Confidence bands will be placed on each class in the trajectory models. Predictions in the outcome scale, ln (IL6) or ln (IL10), will be computed using a Monte Carlo approach with 2,000 draws. Latent class mixture models will be created in each of the two cohorts as well as in the increasing/ decreasing subsets. In the overall analyses, time will be modeled as actual and ln (hours+1). In, the increasing/

decreasing analyses, time will only be modeled as ln (hours+1). When modeling ln (hours+1), a grid search function will be used to find the best set of initial conditions. Grid search is derived from the Expectation Maximization (EM) technique and uses the parameters obtained from the best log-likelihood of a one class model after *m* iterations, in this case 20, and uses them as the initial values for the estimation of parameters in the final, *g* class, model (Proust-Lima et al., n.d.).

In each of the trajectory models, 60 day all-cause mortality will be calculated in each of the classes as the number of individuals who died at or before 60 days from any cause out of the number of individuals with a confirmed death status at 60 days. After discussion with a clinician, it was determined that the mortality rates are similar for those with a confirmed and those without a confirmed death status at 60 days. Therefore there is no bias in removing these individuals from the calculation. Mortality rates will also be compared between increasing and decreasing groups of IL6 and IL10 using a chi square test of proportions. It is suspected that those that increase initially will be caught earlier in the disease process and will have better mortality rates given that treatment began earlier.

In order to investigate mortality patterns in the classes of IL6 and IL10, 60 day all-cause mortality will be calculated in all combinations of IL6 and IL10 classes formed from the overall analysis of the whole cohort. These classes will be ranked from low to high based on the baseline cytokine value in the trajectory and the information will be presented in a table. In order to analyze this table, pairwise chi-square tests of proportions with Bonferroni corrections for multiple comparisons will be computed on the mortality rates between classes in a particular column or row of the table.

3.0 RESULTS

3.1 PATIENTS

The ProCESS trial enrolled 1,351 individuals. Ten of the 1,351 patients asked to be removed from the study leaving a total of 1,341 patients for analysis. Of these 1,341 patients, 638 had at least 1 time point measured, 617 had at least 2 time points measured, 563 had at least 3 time points measured and 167 had all four time points measured as seen in the consort table in Figure 1.

After defining subgroups, the whole cohort group had 638 patients with at least one IL6 and IL10 measurement. Of these 638, 319 are decreasing IL10 and 159 are increasing IL10. Of the 638, 381 have decreasing IL6 and 202 have increasing IL6. The complete measurement cohort group has 549 patients with the first three time points or all four time points, 278 of which are decreasing IL10 and 126 have increasing IL10. Of the 549, 335 are decreasing IL6 and 169 are increasing IL6.

Table 1. shows the baseline and demographic values compared in each of the two main analysis cohorts as well as the group defined as the incomplete measurement cohort. All baseline characteristics are similar statistically between whole cohort and the complete measurement cohort with the exception of baseline and 6 hour ln (IL6) and ln (IL10) values, baseline lactate and 60 day all-cause mortality. Cytokine measurements are higher in the whole cohort as compared to the complete measurement cohort. Baseline lactate values are also higher in the whole cohort. When considering mortality, 60 day all-cause mortality is higher in the whole cohort as well.

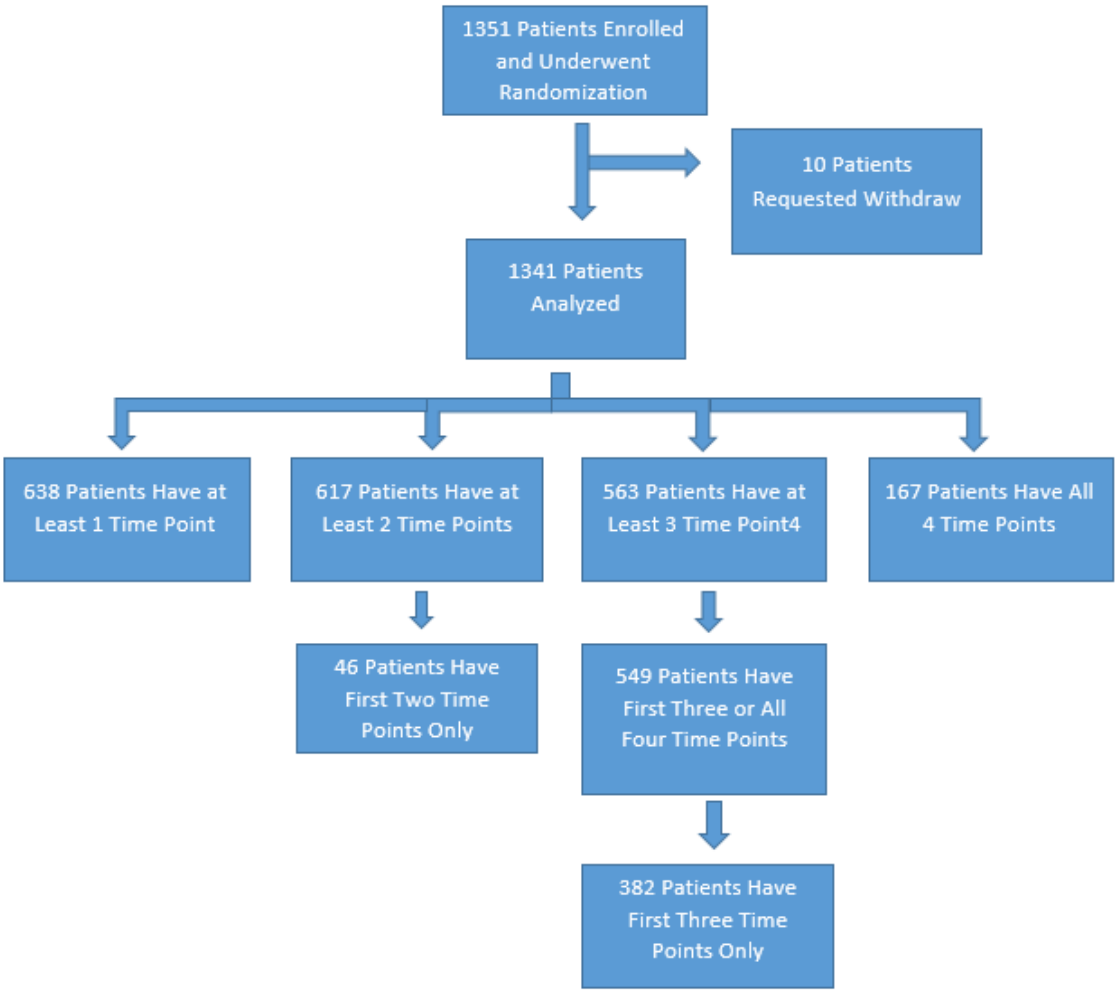

Figure 1. Consort Table

Table 1. Baseline Characteristics between Groups[1] [2]

	WHOLE COHORT (N=638)	COMPLETE MEASUREMENT COHORT (N=549)	INCOMPLETE MEASUREMENT COHORT[3] (N=88)	P-VALUE[4]
BASELINE LN (IL6)[5]	6.13±2.60	6.05±2.53	6.77±2.99	0.0254
BASELINE LN (IL10)[6]	3.63±1.95	3.55±1.89	4.20±2.30	0.0078
6 HOUR LN (IL6)[7]	5.68±2.44	5.61±2.30	6.43±3.37	0.0118
6 HOUR LN (IL10)[8]	3.27±1.75	3.22±1.68	3.75±2.28	0.0249
MALE	371(58.15)	326(59.38)	44(50.00)	0.0978
AGE[9] – YEARS	59.8±15.4	60±15.6	59.2±14.0	0.6821
RACE				0.9422
BLACK	146(22.9)	127(23.1)	19(21.6)	
WHITE	440(69.0)	377(68.7)	62(70.5)	
OTHER	52(8.15)	45(8.20)	7(7.95)	
60 DAY MORTALITY[10]	165/573 (28.8)	125/492 (25.4)	40/81 (49.4)	<0.0001
BASELINE APACHE II SCORE	20.8±7.74	20.6±7.56	22.0±8.75	0.1114
MECHANICAL VENTILATION[11]	243(38.2)	202(36.8)	41(47.1)	0.0654
PRERANDOMIZATION VASOPRESSOR	116(18.2)	100(18.2)	16(18.2)	0.9940
RANDOMIZATION TO 6 HOUR VASOPRESSOR	336(52.7)	289(52.6)	47(53.4)	0.8934
BASELINE SOFA SCORE	7.14±3.62	7.06±3.58	7.64±3.86	0.1687
SEPTIC BY DEFINITION[12]	623(97.7)	534(97.3)	88 (100.0)	0.1166
BASELINE SBP	101±29.6	101±29.4	102±29.2	0.8409
BASELINE DBP	59.1±20.0	59.2±19.7	58.2±19.8	0.6446
BASELINE HEART RATE	112±24.4	111±24.5	116±22.3	0.1335
BASELINE TEMPERATURE (C^o)[13]	37.3±1.59	37.3±1.61	37.4±1.53	0.6362
BASELINE RESPIRATORY RATE[14]	22.6±7.30	22.4±7.06	23.7±8.56	0.1114
BASELINE LACTATE[15]	4.66±3.18	4.53±3.13	5.44±3.38	0.0135
BASELINE WBC[16]	15.8±11.2	15.6±10.4	16.5±14.8	0.6105

[1] Plus/minus values are mean ± standard deviation

[2] Unless otherwise notes, categorical variables are presented as Number (%)

[3] The incomplete measurement cohort plus the complete measurement cohort does not equal the total in the whole cohort. This is a consequence of 1 patient with Il6 values that qualify them as a complete cohort and only one Il10 time point measured.

[4] P-values are calculated using t-tests for numeric variables and chi-squared tests for categorical variables between the complete cohort and incomplete cohorts.

[5] Information on baseline ln (Il6) was missing for 16 patients in the whole cohort group and 16 patients in the incomplete measurement cohorts.

[6] Information of baseline ln (Il10) was missing for 17 patients in the whole cohort group and 16 patients in the incomplete measurement cohort.

[7] Information on 6 hour ln (IL6) was missing for 27 patients in the whole cohort group and 27 patients in the incomplete measurement cohort.

[8] Information on 6 hour ln (IL10) was missing for 27 patients in the whole cohort group and 27 patients in the incomplete measurement cohort.

[9] Information on age is missing for 11 patients in the whole cohort, 8 in the complete measurement cohort and 3 in the incomplete measurement cohort.

[10] Number/Total (%) - Information on mortality at 60 days is not confirmed for 109 patients in the whole cohort group, 57 in the complete measurement cohort and 1 in the incomplete measurement cohort. These individuals were removed from the analysis.

[11] Information on mechanical ventilation was missing for 1 patients in the whole cohort group and 1 patient in the incomplete measurement cohort.

[12] Sepsis is defined as an acute change in SOFA score of 2 or more points.

[13] Information on Baseline Temperature was missing for 8 patients in the whole cohort group, 5 patients in the complete measurement cohort and 3 patients in the incomplete measurement cohort.

[14] Information on Baseline Respiratory Rate was missing for 1 patients in the whole cohort group and 1 patients in the complete measurement cohort group.

[15] Information on Baseline Lactate was missing for 18 patients in the whole cohort group, 15 patients in the complete measurement cohort and 3 patients in the incomplete measurement cohort.

[16] Information on Baseline WBC was missing for 336 patients in the whole cohort group, 293 patients in the complete measurement cohort and 42 patients in the incomplete measurement cohort.

3.2 SURVIVAL

The figure below shows the product limit survival estimates of the whole, complete measurement and incomplete measurement cohorts. The log rank test statistic comparing the complete measurement and incomplete measurement cohorts has a chi-squared test statistic of 31.64 with a p-value of <0.0001 indicating that the two survival curves are statistically different.

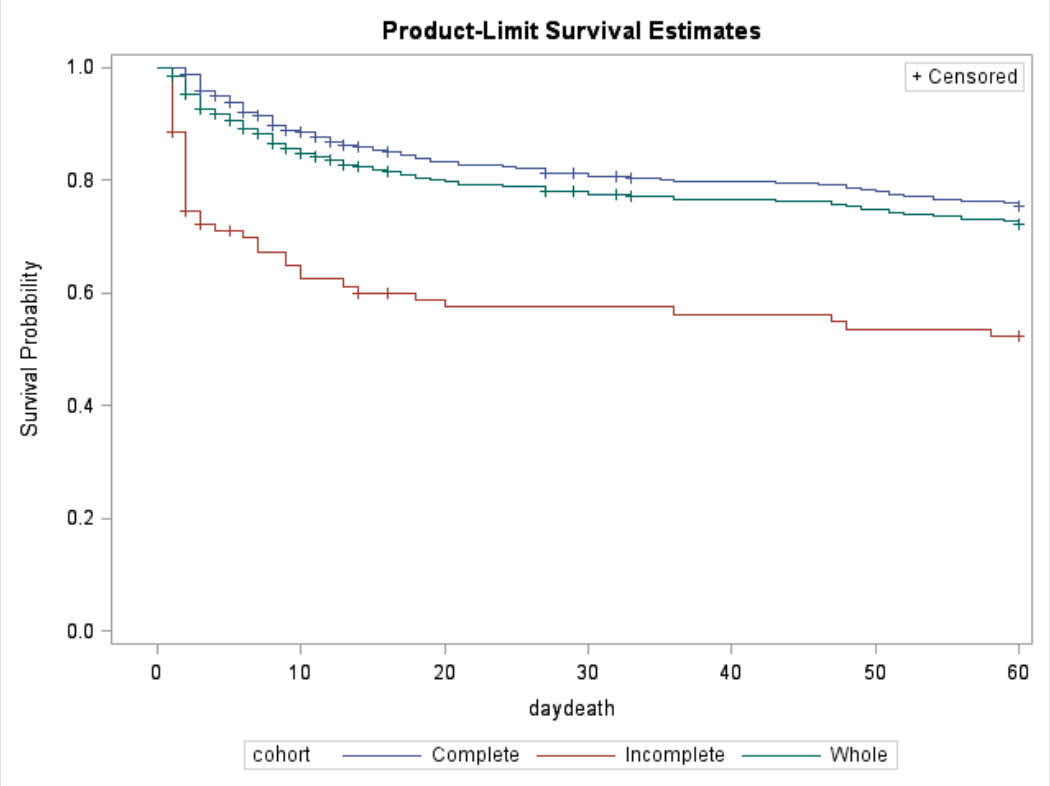

Figure 2. Survival Estimates between Whole, Complete Measurement and Incomplete Measurement Cohorts

3.3 LATENT CLASS DETERMINATION

After running all of the overall models, it was apparent that most of the models would have 3, 4 or 5 classes with 4 classes being dominant. In order to preserve consistency between models and allow for easier clinical interpretation, all models were modeled with 4 classes. The only exception is the model for IL10 in the complete measurement cohort using ln (hours+1) times. For these data, the 4 class model included a fourth class which contained no patients. Therefore, a 3 class model was used.

After running the overall analysis in the whole and complete measurement cohorts using both actual and ln (hours+1) time, a spaghetti plot of the outcome was plotted against time. Comparing the trajectory classes and the spaghetti plots revealed that modeling time as ln (hours+1) better reflected the data. Models were also considered using rank times (1, 2, 3, 4) but were abandoned given the difficulty of clinical interpretation when data is measured at times other than 0, 6, 24 and 72 hours. When modeling with actual times, some classes took a parabolic shape that was not supported by the data. Only models using ln (hours+1) time will be discussed.

For the increasing and decreasing analysis, models were determined using a combination of BIC, class size, as well as clinical significance. Considering BIC and removing a class size of less than 8% produced 2 class models in all IL6 and IL10, increasing and decreasing models. Adding clinical interpretation allowed a third class in the decreasing analysis of IL6 in all subgroups. All models use time as ln (hours+1) for the reasons stated above.

Sex, age, and race were only significant in a few of the models, so all covariates were removed to preserve consistency. In the figures for models one through twelve below, the 95% confidence intervals are shown around all class trajectories and each y-axis is shown on the actual cytokine scale, as opposed to the natural log of the cytokine measurement, in order to be more recognizable to clinicians. When modeling ln (hours+1), the x-axis has the actual hour measurement at the location of the ln (hours+1) time point.

3.4 OVERALL ANALYSIS IN WHOLE COHORT

The overall analysis of IL6 using ln (hours+1) time in the whole cohort is considered Model 1. Parameter estimates for Model 1 can be found in the table below. Class 1 is represented by a lower initial cytokine value with slow decline. Class 2 is represented by the highest initial cytokine value and later decline. Class 3 is represented by low and steady cytokine values. Class 4 is represented by higher initial cytokine values with quicker decline. In this model, 44.2% of the patients belong to Class 1, 1.88% of the patients belong to Class 2, 48.9% of the patients belong to Class 3 and 5.02% of the patients belong to Class 4. A majority of the patients belong to Class 3. Mortality is as follows: Class 1 28.35%, Class 2 81.82%, Class 3 27.30% and Class 4 26.92%. A figure of the trajectory classes is shown below.

The overall analysis of IL10 using ln (hours+1) times in the whole cohort is considered Model 2. Parameter estimates for Model 2 can be found in the table below. Class 1 is represented by a higher initial cytokine value with slow decline. Class 2 is represented by a moderate initial cytokine value and slow decline. Class 3 is represented by low and steady cytokine values. Class 4 is represented by the highest initial cytokine value with quicker decline. In this model 9.09% of the patients belong to Class 1, 20.06% of the patients belong to Class 2, 65.52% of the patients belong to Class 3 and 5.33% of the patients belong to Class 4. A majority of the patients belong to Class 3. Mortality is as follows: Class 1 48.15%, Class 2 21.93%, Class 3 27.01% and Class 4 41.94%. A figuring comparing of the trajectory classes is shown below.

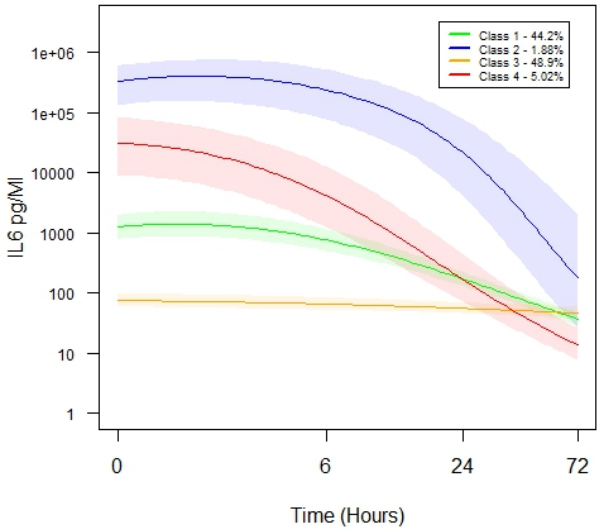

Figure 3. Overall Analysis of IL6 in Whole Cohort Using Ln (hours+1) Times – Model 1

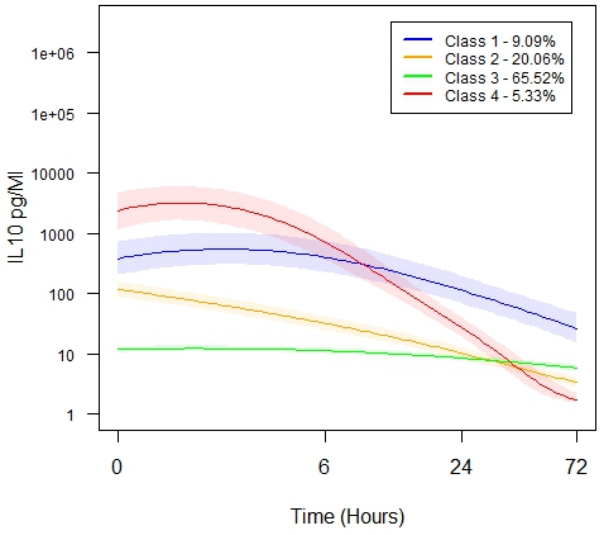

Figure 4. Overall Analysis of IL10 in Whole Cohort Using Ln (hours+1) Times – Models 2

Table 2. Parameter Estimates for Model 1

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	7.26413	0.88566	8.202	<0.0001
CLASS 3 INTERCEPT	-3.1761	0.23772	-13.361	<0.0001
CLASS 4 INTERCEPT	3.40528	0.71302	4.776	<0.0001
TIME CLASS 1	0.36122	0.09507	3.799	0.00015
TIME CLASS 2	1.21214	0.56797	2.134	0.03283
TIME CLASS 3	-0.03986	0.08344	-0.478	0.63285
TIME CLASS 4	-0.2318	0.31486	-0.736	0.4616
TIME SQUARED CLASS 1	-0.32002	0.02873	-11.138	<0.0001
TIME SQUARED CLASS 2	-0.7912	0.1827	-4.33	0.00001
TIME SQUARED CLASS 3	-0.03413	0.0233	-1.465	0.14297
TIME SQUARED CLASS 4	-0.46557	0.073	-6.378	<0.0001

Table 3. Parameter Estimates for Model 2

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	-1.37894	0.41123	-3.353	0.0008
CLASS 3 INTERCEPT	-4.91086	0.35577	-13.804	<0.0001
CLASS 4 INTERCEPT	1.8515	0.53788	3.442	0.00058
TIME CLASS 1	0.73912	0.19786	3.736	0.00019
TIME CLASS 2	-0.71192	0.16576	-4.295	0.00002
TIME CLASS 3	0.14351	0.06728	2.133	0.03291
TIME CLASS 4	0.99133	0.27537	3.6	0.00032
TIME SQUARED CLASS 1	-0.36791	0.05345	-6.884	<0.0001
TIME SQUARED CLASS 2	-0.14199	0.03885	-3.655	0.00026
TIME SQUARED CLASS 3	-0.09596	0.01761	-5.448	<0.0001
TIME SQUARED CLASS 4	-0.8283	0.08176	-10.131	<0.0001

3.5 OVERALL ANALYSIS IN COMPLETE MEASUREMENT COHORT

The overall analysis of IL6 using ln (hours+1) times in the complete measurement cohort is considered Model 3. Parameter estimates for Model 3 can be found in the table below. Class 1 is represented by a lower initial cytokine value that increases and then decreases quickly. Class 2 is represented by low and steady cytokine values. Class 3 is represented by moderate initial cytokine values with slow decline. Class 4 is represented by higher initial cytokine values that decline slowly at first then quicker as time progresses. In this model 0.18 % of the patients belong to Class 1, 45.9% of the patients belong to Class 2, 47.54% of the patients belong to Class 3 and 6.38% of the patients belong to Class 4. A majority of the patient belong to Class 3. Mortality is as follows: Class 1 0%, Class 2 23.81%, Class 3 20.69% and Class 4 31.43%. A figuring showing the trajectory classes is shown below.

The overall analysis of IL10 using ln (hours+1) times in the complete measurement cohort is considered Model 4. Parameter estimates for Model 4 can be found in the table below. Class 1 is represented by the highest cytokine value with slow decline. Class 2 is represented by low and steady cytokine values. Class 3 is represented by high initial cytokine values that decline quickly. In this model 6.01% of the patients belong to Class 1, 69.03% of the patients belong to Class 2 and 24.95% of the patients belong to Class 3. A majority of the patients belong to Class 2. Mortality is as follows: Class 1 51.61%, Class 2 25.07%, and Class 3 19.67%. A figuring showing the trajectory classes is shown below.

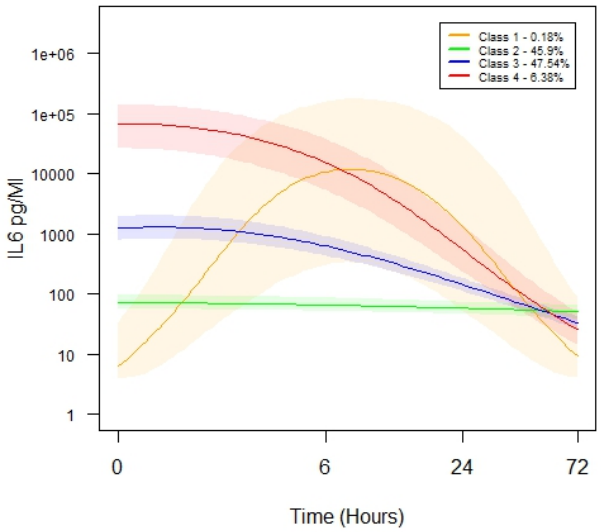

Figure 5. Overall Analysis of IL6 in Complete Measurement Cohort Using Ln (hours+1) Times – Model 3

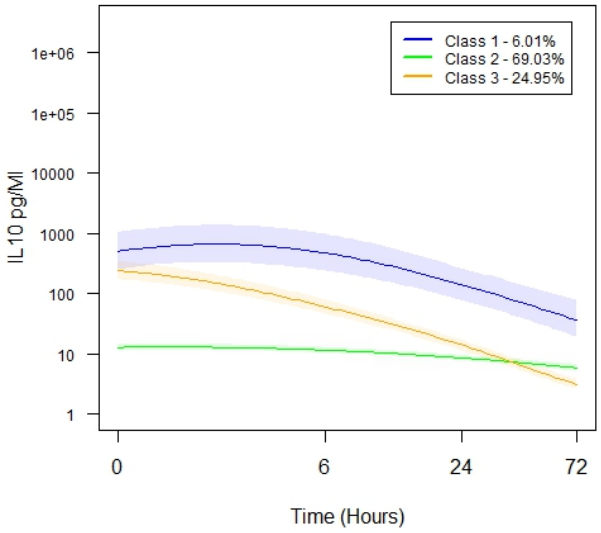

Figure 6. Overall Analysis of IL10 in Complete Measurement Cohort Using Ln (hours+1) Times – Models 4

Table 4. Parameter Estimates for Model 3

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	4.93858	1.88285	2.623	0.00872
CLASS 3 INTERCEPT	8.21856	1.89139	4.345	0.00001
CLASS 4 INTERCEPT	12.72625	2.00175	6.358	<0.0001
TIME CLASS 1	9.64188	1.1511	8.376	<0.0001
TIME CLASS 2	-0.02286	0.08747	-0.261	0.79385
TIME CLASS 3	0.23732	0.09473	2.505	0.01224
TIME CLASS 4	0.06125	0.26127	0.234	0.81464
TIME SQUARED CLASS 1	-2.19693	0.25501	-8.615	<0.0001
TIME SQUARED CLASS 2	-0.03079	0.02455	-1.254	0.20968
TIME SQUARED CLASS 3	-0.3066	0.02904	-10.557	<0.0001
TIME SQUARED CLASS 4	-0.536	0.0705	-7.602	<0.0001

Table 5. Parameter Estimates for Model 4

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	-4.56272	0.36312	-12.565	<0.0001
CLASS 3 INTERCEPT	-0.675	0.40579	-1.663	0.09623
TIME CLASS 1	0.53142	0.2322	2.289	0.0221
TIME CLASS 2	0.08182	0.06711	1.219	0.22278
TIME CLASS 3	-0.28908	0.12668	-2.282	0.02249
TIME SQUARED CLASS 1	-0.28447	0.06253	-4.549	0.00001
TIME SQUARED CLASS 2	-0.08569	0.01771	-4.839	<0.0001
TIME SQUARED CLASS 3	-0.27181	0.03244	-8.378	<0.0001

3.6 INCREASING/DECREASING ANALYSIS IN WHOLE COHORT

The increasing analysis of IL6 in the whole cohort using ln (hours+1) times is considered Model 5. Parameter estimates for Model 5 can be found in the table below. Class 1 is represented by a higher initial cytokine value with slow increase and then decrease. Class 2 is represented by lower initial cytokine value and even smaller increase and decrease than Class 1. The figure below shows this 2 class trajectory model. In this model 52.48% of the patients belong to Class 1 and 47.52% of the patients belong to Class 2. A majority of the patients belong to Class 1. Mortality is as follows: Class 1 37.36% and Class 2 34.78%.

The increasing analysis of IL10 in the whole cohort using ln (hours+1) times is considered Model 6. Parameter estimates for Model 6 can be found in the table below. Class 1 is represented by lower initial cytokine value with a small increase and then decrease. Class 2 is represented by a higher initial cytokine value with a larger increase and decrease than Class 1. The figure below shows this 2 class trajectory model. In this model 61.64% of the patients belong to Class 1 and 38.36% of the patients belong to Class 2. A majority of the patients belong to Class 1. Mortality is as follows: Class 1 34.07% and Class 2 42.31%.

The decreasing analysis of IL6 in the whole cohort using ln (hours+1) times is considered Model 7. Parameter estimates for Model 7 can be found in the table below. Class 1 is represented by moderate initial values and moderate decline. Class 2 is represented by low initial value that almost remains steady. Class 3 is represented by high initial value and slow decline followed by more rapid decline. The figure below shows this 3 class trajectory model. In this model 37.53% of the patients belong to Class 1, 56.17% of the patients belong to Class 2 and 6.3% of the patients belong to Class 3. A majority of the patients belong to Class 2. Mortality is as follows: Class 1 22.66%, Class 2 24.74% and Class 3 59.09%.

The decreasing analysis of IL10 in the whole cohort using ln (hours+1) times is considered Model 8. Parameter estimates for Model 8 can be found in the table below. Class 1 is represented by low and steady cytokine values. Class 2 is represented by higher initial values that decline slowly. The figure below shows this 2 class trajectory model. In this model 78.06% of the patients belong to Class 1 and 21.94% of the patients belong to Class 2. A majority of the patients belong to Class 1. Mortality is as follows: Class 1 27.71% and Class 2 31.25%.

When considering Il6, the overall mortality in the increasing group was 36.07% and the overall mortality in the decreasing group was 26.16%. The proportion of patients dying at 60 days from any cause in the increasing group is significantly different than the decreasing group with a p-value of 0.0177. When considering Il10, the overall mortality in the increasing group was 37.06% and the overall mortality in the decreasing group was 28.47%. The proportion of patients dying at 60 days from any cause in the increasing group was not significantly different from the decreasing group with a p-value of 0.0691.

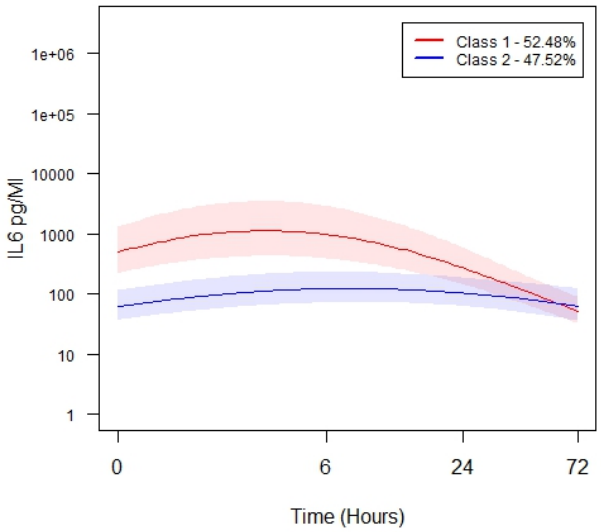

Figure 7. Increasing Analysis of IL6 in Whole Cohort Using Ln (hours+1) Times – Model 5

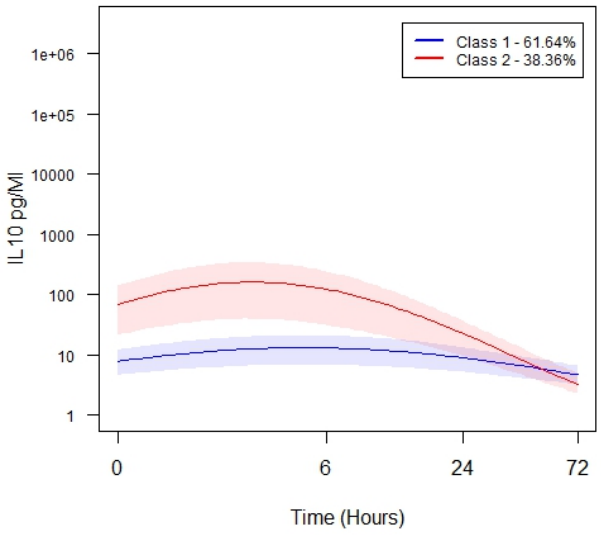

Figure 8. Increasing Analysis of IL10 in Whole Cohort Using Ln (hours+1) Times – Model 6

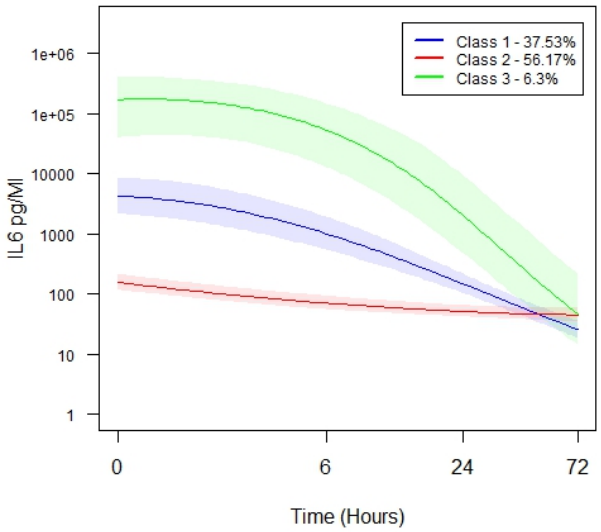

Figure 9. Decreasing Analysis of IL6 in Whole Cohort Using Ln (hours+1) Times – Model 7

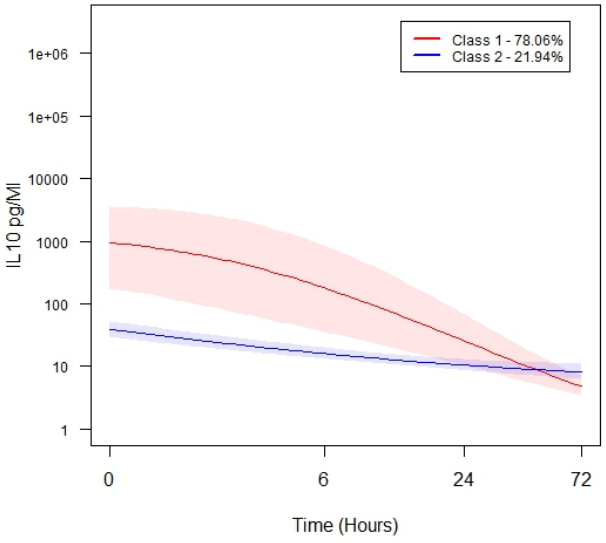

Figure 10. Decreasing Analysis of IL10 in Whole Cohort Using Ln (hours+1) Times – Model 8

Table 6. Parameter Estimates for Model 5

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	-2.57337	0.58335	-4.411	0.00001
TIME CLASS 1	1.22398	0.17978	6.808	<0.0001
TIME CLASS 2	0.90315	0.14756	6.12	<0.0001
TIME SQUARED CLASS 1	-0.44379	0.06055	-7.329	<0.0001
TIME SQUARED CLASS 2	-0.212	0.04358	-4.865	<0.0001

Table 7. Parameter Estimates for Model 6

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	2.86655	0.39863	7.191	<0.0001
TIME CLASS 1	0.8766	0.14221	6.164	<0.0001
TIME CLASS 2	1.4567	0.19131	7.614	<0.0001
TIME SQUARED CLASS 1	-0.25425	0.03787	-6.714	<0.0001
TIME SQUARED CLASS 2	-0.58388	0.06024	-9.693	<0.0001

Table 8. Parameter Estimates for Model 7

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	-4.03315	0.35505	-11.359	<0.0001
CLASS 3 INTERCEPT	5.16057	0.96934	5.324	<0.0001
TIME CLASS 1	-0.21468	0.14974	-1.434	0.15166
TIME CLASS 2	-0.71197	0.10185	-6.99	<0.0001
TIME CLASS 3	0.36568	0.3564	1.026	0.30488
TIME SQUARED CLASS 1	-0.33486	0.05155	-6.496	<0.0001
TIME SQUARED CLASS 2	0.05366	0.03041	1.765	0.07758
TIME SQUARED CLASS 3	-0.69001	0.10522	-6.558	<0.0001

Table 9. Parameter Estimates for Model 8

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	-3.90296	0.92644	-4.213	0.00003
TIME CLASS 1	-0.37277	0.26982	-1.382	0.16711
TIME CLASS 2	-0.74246	0.09728	-7.632	<0.0001
TIME SQUARED CLASS 1	-0.31945	0.10733	-2.976	0.00292
TIME SQUARED CLASS 2	0.03455	0.02751	1.256	0.20925

3.7 INCREASING/DECREASING ANALYSIS IN COMPLETE MEASUREMENT COHORT

The increasing analysis of IL6 in the complete measurement cohort using ln (hours+1) times is considered Model 9. Parameter estimates for Model 9 can be found in the table below. Class 1 is represented by lower initial cytokine value with a small increase and then decrease. Class 2 is represented by a higher initial cytokine value with a larger increase and decrease than Class 1. The figure below shows this 2 class trajectory model. In this model 47.34% of the patients belong to Class 1 and 52.66% of the patients belong to Class 2. A majority of the patients belong to Class 2. Mortality is as follows: Class 1 35.53% and Class 2 33.33%.

The increasing analysis of IL10 in the complete measurement cohort using ln (hours+1) times is considered Model 10. Parameter estimates for Model 10 can be found in the table below. Class 1 is represented by lower initial cytokine values with a small increase and then decrease. Class 2 is represented by a higher initial cytokine value with a larger increase and decrease than Class 1. The figure below shows this 2 class trajectory model. In this model 63.49% of the patients belong to Class 1 and 36.51% of the patients belong to Class 2. The majority of patients belong to Class 1. Mortality is as follows: Class 1 32.88% and Class 2 35.00%.

The decreasing analysis of IL6 in the complete measurement cohort using ln (hours+1) times is considered Model 11. Parameter estimates for Model 11 can be found in the table below. Class 1 is represented by moderate initial values and moderate decline. Class 2 is represented by a low initial value that remains steady. Class 3 is represented by high initial value and slow decline followed by more rapid decline. The figure below shows this 3 class trajectory model. In this model 42.39% of the patients belong to Class 1, 51.04% of the patients belong to Class 2 and 6.57% of

the patients belong to class 3. A majority of the patients belong to Class 2. Mortality is as follows: Class 1 18.4%, Class 2 22.15% and Class 3 45.00%.

The decreasing analysis of IL10 in the complete measurement cohort with ln (hours+1) times is considered Model 12. Parameter estimates for Model 12 can be found in the table below. Class 1 is represented by low and steady cytokine values. Class 2 is represented by higher initial cytokine values that decline slowly. The figure below shows this 2 class trajectory model. In this model 71.58% of the patients belong to Class 1 and 28.42% of the patients belong to Class 2. A majority of the patients belong to Class 1. Mortality is as follows: Class 1 26.09% and Class 2 23.61%.

When considering Il6, the overall mortality in the increasing group was 34.44% and the overall mortality in the decreasing group was 22.11%. The proportion of patients dying at 60 days from any cause in the increasing group is significantly different from the decreasing group with a p-value of 0.0049. When considering Il10, the overall mortality in the increasing group was 33.63% and the overall mortality in the decreasing group was 25.39%. The proportion of patients dying at 60 days from any cause in the increasing group was not significantly different from the decreasing group with a p-value of 0.1040.

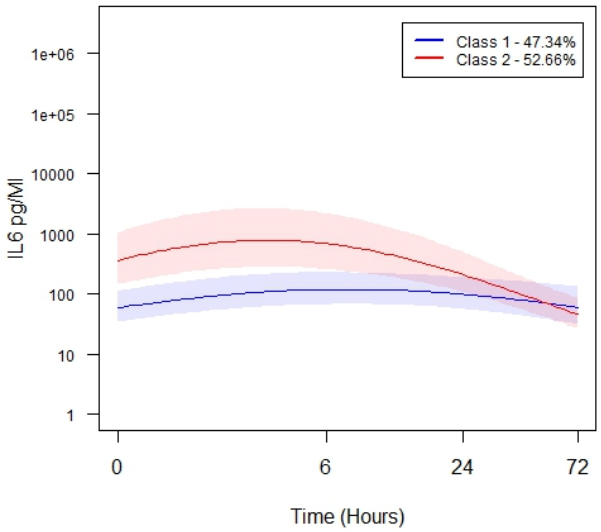

Figure 11. Increasing Analysis of IL6 in Complete Measurement Cohort Using Ln (hours+1) Times – Model 9

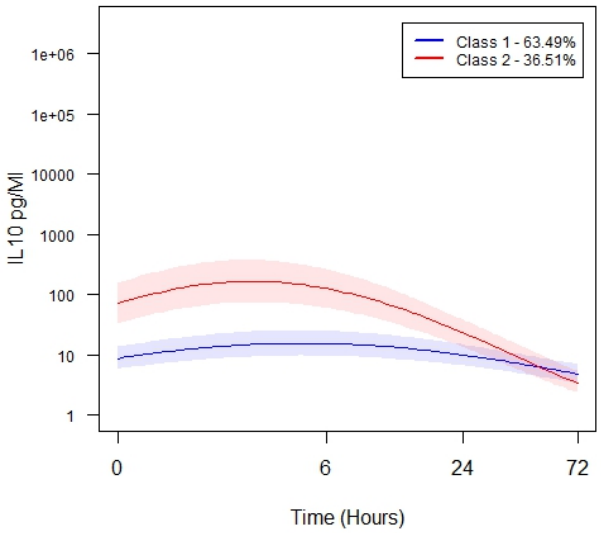

Figure 12. Increasing Analysis of IL10 in Complete Measurement Cohort Using Ln (hours+1) Times – Model 10

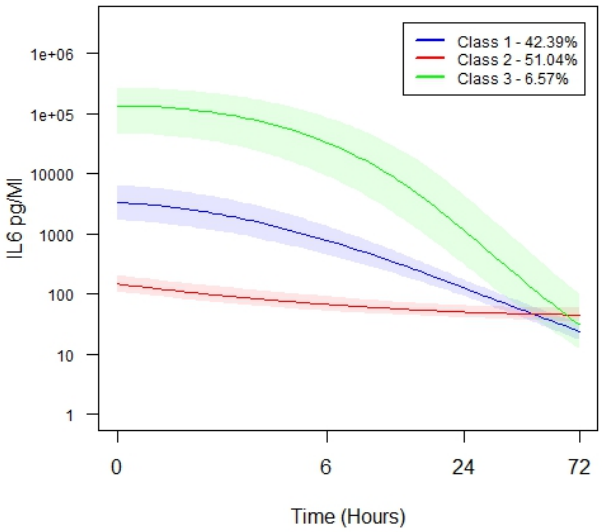

Figure 13. Decreasing Analysis of IL6 in Complete Measurement Cohort Using Ln (hours+1) Times – Model 11

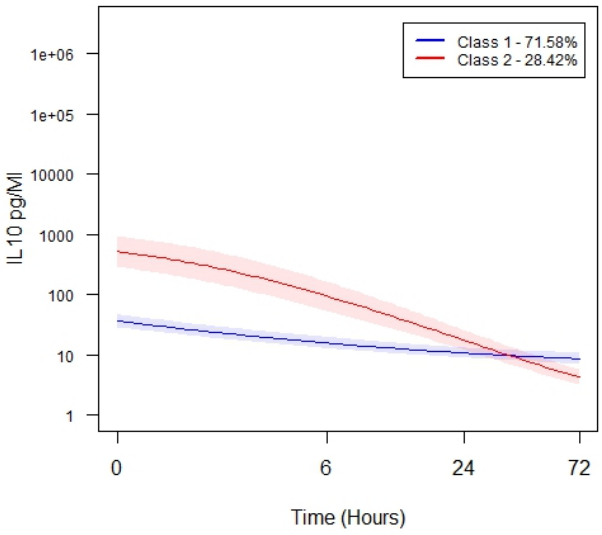

Figure 14. Decreasing Analysis of IL10 in Complete Measurement Cohort Using Ln (hours+1) Times – Model 12

Table 10. Parameter Estimates for Model 9

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	2.29886	0.66318	3.466	0.00053
TIME CLASS 1	0.92019	0.17021	5.406	<0.0001
TIME CLASS 2	1.1843	0.1884	6.286	<0.0001
TIME SQUARED CLASS 1	-0.21823	0.05155	-4.234	0.00002
TIME SQUARED CLASS 2	-0.42738	0.06196	-6.898	<0.0001

Table 11. Parameter Estimates for Model 10

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	2.60822	0.42978	6.069	<0.0001
TIME CLASS 1	0.92006	0.155	5.936	<0.0001
TIME CLASS 2	1.39951	0.20624	6.786	<0.0001
TIME SQUARED CLASS 1	-0.2688	0.0429	-6.265	<0.0001
TIME SQUARED CLASS 2	-0.55909	0.06358	-8.794	<0.0001

Table 12. Parameter Estimates for Model 11

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	-3.77528	0.35546	-10.621	<0.0001
CLASS 3 INTERCEPT	5.29336	0.82155	6.443	<0.0001
TIME CLASS 1	-0.25107	0.13387	-1.875	0.06073
TIME CLASS 2	-0.72525	0.11031	-6.574	<0.0001
TIME CLASS 3	0.01981	0.37843	0.052	0.95824
TIME SQUARED CLASS 1	-0.31137	0.04566	-6.819	<0.0001
TIME SQUARED CLASS 2	0.06368	0.03347	1.902	0.05714
TIME SQUARED CLASS 3	-0.63789	0.10127	-6.299	<0.0001

Table 13. Parameter Estimates for Model 12

	COEFFICIENT	STANDARD ERROR	WALD TEST STATISTIC	P-VALUE
CLASS 1 INTERCEPT (NOT ESTIMATED)	0	-	-	-
CLASS 2 INTERCEPT	3.2259	0.37979	8.494	<0.0001
TIME CLASS 1	-0.73392	0.09918	-7.4	<0.0001
TIME CLASS 2	-0.52825	0.16825	-3.14	0.00169
TIME SQUARED CLASS 1	0.04667	0.02519	1.853	0.06387
TIME SQUARED CLASS 2	-0.25346	0.04786	-5.295	<0.0001

3.8 MORTALITY PATTERNS

The table below shows the 60 day all-cause mortality in each IL6/IL10 category. This table uses the results of the overall trajectory analyses in the whole cohort using ln (hours+1) times. Classes are ordered from low baseline cytokine value to high baseline cytokine value according to the appropriate trajectory analysis. As seen below, the highest mortality is seen in patients placed in IL6 class 2 and IL10 class 1.

After running the chi-square tests, it was determined that the mortality rates for Class 2 differ statistically from the mortality rates in Class 3, Class 1 and Class 4 when considering IL6. Mortality rates for Class 1 differ from Class 3 and Class 2 when considering IL10.

Table 14. IL6/IL10 Mortality Comparison*

	IL6 Class 3	IL6 Class 1	IL6 Class 4	IL6 Class 2	Total
IL10 Class 3	260 (28.39%)	155 (25.19%)	2 (0.00%)	1 (0.00%)	418 (27.01%)
IL10 Class 2	39 (17.65%)	76 (25.35%)	13 (11.11%)	0 (NA)	128 (21.93%)
IL10 Class 1	12 (36.36%)	37 (42.86%)	2 (50.00%)	7 (100.00%)	58 (48.15%)
IL10 Class 4	1 (0.00%)	14 (38.46%)	15 (38.46%)	4 (75.00%)	34 (41.94%)
Total	312 (27.30%)	282 (28.35%)	32 (26.92%)	12 (81.82%)	638 (28.80%)

* Values in the table are Total N (Mortality Rate)

4.0 DISCUSSION

In this study, both the whole and complete measurement cohorts looked similar clinically although the high mortality in the incomplete measurement group drove up the mortality in the whole cohort making it statistically different from the mortality rate in the complete measurement cohort. It is also important to note that many individuals in the incomplete measurement group die before 3 days. Not only are the individuals in this group sicker, but they are also dying earlier than in the other two cohorts.

In regards to modeling, the decreasing analysis of IL6 in all subgroups was modeled with three groups. The reason for this additional group is based on the clinical understanding that IL6 would have a flat group, a slowly decreasing group and a rapidly decreasing group. The resulting models confirmed these beliefs.

In the overall analysis of the whole cohort for Il6, the highest mortality is in the highest initial value class. In the overall analysis of the complete measurement cohort for Il6, the highest mortality is also in the highest initial value class. The shape of the resulting classes from the whole and complete measurement cohorts are very similar with the addition of the rising class in the complete measurement cohort. It is important to note the extremely small number of individuals being placed into this increasing class.

In the overall analysis of the whole cohort for IL10, Class 1 has a higher mortality rate then Class 4 even though Class 4 has a higher initial IL10 value. This suggests that the higher mortality in Class 1 is driven by the high initial value as well as the slower decline over time. This idea is reinforced once again in the overall analysis of the complete measurement cohort. Even though Class 1 and Class 3 have similar initial values, the higher mortality is in the class that declines

slower. The trajectory classes for IL10 in both the whole and complete measurement cohorts are extremely similar in shape and interpretation.

In the increasing/decreasing analysis, the highest mortality was in the group with the highest initial cytokine value with two exceptions. These exceptions are both in the complete measurement cohort and are seen in the Il6 increasing model and Il10 decreasing model. In both of these models the lower of the two curves has the lower mortality. Even though it is lower, the mortality levels in the two groups are very similar. Models of increasing/decreasing subsets look very similar between cohorts.

When looking at IL6 in both cohorts, there is a statistically significant difference in mortality between those who increase from 0 to 6 and those who decrease from 0 to 6. It was originally suspected that those who initially rise were caught earlier and would have better outcomes but this is not the case. Instead, those who rise initially have higher mortality. This indicates that these individuals are sicker. Although it was not significant in IL10, the same trend is present.

When comparing classes of IL6 and IL10 simultaneously, we see the same trend presented in the univariate interpretations. In all combinations of IL10 with IL6 Class 4, a higher initial level of IL6 does not indicate highest mortality. Mortality is much lower in Il6 Class 4 than Class 2 because of the quicker drop. We see the highest mortality not in the highest IL6/IL10 class but in the highest IL6 class crossed with the third highest IL10 class but this is not strong support given the small number of individuals in this category.

All in all, the analyses presented support the following key findings:

- Mortality in IL6 and IL10 is not only based on initial values
- Rate of decent plays a key role in mortality
- Increasing cytokine values from time 0 to time 6 indicate worse outcomes
- Interpretation from both cohorts presents similar findings

These results further qualify the results of Kellum et al who state that the highest mortality can be found in those with high/high combinations of IL6 and IL10. The difference in our results can be attributed to the completeness of the ProCESS data set and the addition of increasing trajectories. As shown in this study and the study by Kellum et al, there is information in longitudinal data.

In regards to bias, it is clear that using either of the cohorts would have produced similar results therefore the survivor bias is not apparent in the complete measurement cohort.

4.1 LIMITATIONS

One of the main limitations of this study lies the determination of classes. Although BIC is an excellent method for choosing the number of classes, it also has its flaws. Using BIC alone can produce classes with as little as 1 or 2 individuals. Looking at the mortality rates in these small classes can be deceiving. Therefore, it is up to the clinician and the statistician to determine the optimal number of classes based on the BIC, while also considering class size and clinical significance.

The data itself is limited in regards to defining time 0. In this study, time 0 is the entrance into the study. Unfortunately, study entrance does not coordinate exactly with onset of disease. Therefore, individuals could have seen a peak in their IL6 or IL10 before arriving at the emergency department.

Another limitation lies in the LCMM program itself. When constructing models, LCMM will produce trajectories of a given polynomial form even if some of the terms are not significant in one of the classes. Although this is not a major concern, it is not optimal. Also, when constructing models, LCMM only allows time to be modeled in polynomial form. Other distribution forms of time may be of interest but are unable to be investigated at this time. Lastly, it is important to note that there is no biological explanation for the spline transform and it was only used to better fit data.

4.2 FUTURE DIRECTION

In the future, it would be of value to find a program that produced multivariate trajectory models in a useful way. Although LCMM does have a multlcmm option that does multivariable analysis, this method was unable to derive mixed classes of high IL6/low IL10. As mentioned previously, time 0 in this study is not reflective of the true start of disease progression, it instead reflects entrance into the study. It would therefore be useful to be able to determine the offset from true time zero for each patient and shift them accordingly. For example, if an individual developed sepsis 6 hours before being admitted into the study, their 0, 6, 24 and 72 hour time points should be shifted to 6, 12, 30 and 78.

4.3 PUBLIC HEALTH SIGNIFICANCE

Given the public health significance of sepsis, understanding the prognosis is extremely important. Previously, having a high IL6 measurement implied a poor prognosis. Our results show that many factors play into the determination of prognosis and patients can be treated accordingly.

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