



**Consulting project Comparing Meso Scale Discovery with Quanterix Simoa in terms
of their performance in detecting Cytokine levels**

By Sunhwa Park

Client: Brett Winters

Advisors: Dr. Steve Marron

Dr. Kai Zhang

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UNC CHAPEL HILL

Statistics and Operations Research

Comparing Meso Scale Discovery with Quanterix Simoa in terms of their performance in detecting Cytokine levels

On behalf of Brett Winters

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Abstract

Measuring cytokine levels from exhaled breath from patients who cannot breathe on their own is important because these cytokines are used for diagnosing toxicity levels and diseases. Two measuring instruments, Meso Scale Discovery (MSD) and Quanterix Simoa(QS), were compared across ten different cytokines. My client wants to find out which instrumentation performs better in terms of detecting cytokine levels, one having larger mean with smaller variation among replicates, and which predictors are driving variables in predicting the level of each cytokine. The results indicate that QS performs better in detecting cytokine levels since its mean score is larger with smaller variance as the results from two statistical methods indicate. First, paired two sample t-test shows that the mean difference between MSD and QS is significantly different from zero since p-value < 0.0001 is smaller than 0.05 with 0.05 significance level, and $t = -13.904$ being negative indicates that QS has larger mean than MSD. Second, mixed effects model results show that variance between replicates in QS which is 0.00138 is found to be smaller than that between replicates of MSD which is 0.01188. CPIS and disease status predictors were found to be marginally significant in predicting variance between MSD and QS as the result of regression analysis indicates. Pneumonia, sepsis and death were found to be significant in predicting cytokine levels while ARDS is marginally significant as the results of regression analysis as well as mixed effect model analysis indicate. Disease variables play an important role in accounting for the variance of cytokine scores in the two subsets of data as PCA analysis indicate.

1. Introduction

Exhaled breath condensate (EBC) contains all sorts of different molecules. These molecules in exhaled breath from intubated patients who cannot breathe on their own are used for diagnosing disease or studying toxicity. The tables below show the list of explanatory variables and response variables. Ten different cytokines were measured by either Meso Scale Discovery(MSD) or Quanterix Simoa(QS). The table 1.1 shows the list of explanatory variables while the table 1.2 shows the list of response variables.

1.1 Explanatory variables.

Name of the variables	Description	Scale
CPIS	Cardiopulmonary Infection Score	1-12 ordinal
CXRAY	Chest X-ray score	1-10 ordinal

PH	indicates how acidic or alkaline a cytokine is	continuous
Pneumonia	Lung disease	binary
ARDS	Acute Respiratory Distress Syndrome	binary
Sepsis	Lung disease	binary
Death	Death in patients	binary

Table1.1 List of explanatory variables.

1.2 Response Variables

Name of the response variables	Description	Scale
MSD16_1, MSD16_2, MSD16_3, MSD16_4	Four repeated MSD scores measured in different wells in 2016	Continuous
MSD18_1, MSD18_2	Two repeated MSD scores measured in different wells in 2018	Continuous
QS18_1, QS18_2	Two repeated QS scores measured in different wells in 2018	Continuous

Table 1.2 The repeated measured response variables

The first objective of this analysis is to find out which instrument performs better in terms of detecting cytokine level. The second objective is to find out whether cytokines in patients with any of these respiratory diseases (sepsis, ARDS and pneumonia) are higher than those without having any of the respiratory diseases. The third objective is to find out whether there is large variability among four repeated measures in both MSD and QS just to detect if both instruments are performing correctly. The fourth objective is to find out whether correlations between each predictors and each cytokine score measured by MSD16 are significantly different from zero. Throughout this report, cytokine scores measured by MSD and QS will be simply called MSD score and QS score for the simplicity. Cytokines are also called interchangeably with cytokine data sets. Even though each cytokine shares the same predictors, they all have different values of MSD scores and its replicates and could not be displayed on one screen for coding. So each cytokine with predictors was treated as one data set.

2- Data overview

There are ten different cytokines which are IFNG, IL-1B, IL-10, IL-12p70, IL-13, IL-2, IL-4, IL-6, IL-8, TNFA. While IL-1B and IL-8 cytokines have both MSD and QS scores, other eight cytokines have only MSD scores. In addition, while IL-1B, IL-8, IL-6 and TNFA cytokines have MSD scores in two different years (2016, 2018), other eight cytokines have MSD only in 2016 as the table2 below shows. The study design has multiple measures for each subject. Cytokine IL-1b and IL-8 scores in year 2018 are repeatedly measured for five consecutive days to capture if there would be any day where the cytokine score is higher or highest, and also every sample within a subject is repeatedly measured on four different wells simultaneously. This would violate the independence

assumption since multiple responses from the same subject and multiple responses from the same sample within a subject cannot be regarded as independent from each other. Every patient has a slightly different average cytokine scores, and this is going to be a distinctive factor that affects cytokine IL-1b and IL-8 scores from the same subject, thus rendering these different cytokine scores inter-dependent rather than independent. Similarly, every sample within a subject has a slightly different average cytokine scores, and this is going to be a distinctive factor that affects cytokine IL-1b and IL-8. The way we dealt with this situation was to add a random effect for subject and a nested random effect for sample to capture different sources of variations of the cytokine scores.

Name of Cytokines	MSD 2016	MSD 2018	QS 2018
IL-1b	Yes	Yes	Yes
IL-2	Yes		
IL-4	Yes		
IL-6	Yes	Yes	
IL-8	Yes	Yes	Yes
IL-10	Yes		
IL-12p70	Yes		
IL-13	Yes		
TNFA	Yes	Yes	
IFNG	Yes		

Table 2: list of response variables for each cytokine

3. Methods & results

In order to find answers to the client's research questions, the six different statistical methods are used as shown in method section: univariate analysis, regression analysis, mixed effect analysis, principal component analysis, logistic regression and correlation analysis.

3.1 Univariate Analysis

3.1a One sample t-test

Two univariate analyses were conducted. First, one sample t-test was used to capture the variance between QS and MSD. In order to do the analysis, a variable called difference of squared difference, $DSD = (MSD18_1 - MSD18_2)^2 - (QS18_1 - QS18_2)^2$ was derived where $MSD18_1$, $MSD18_2$, $QS18_1$, and $QS18_2$ represent repeated measures in two different wells in 2018. Since none of these variables met the normality assumption, each of them was log transformed. After the log transformation, two of these four variables became approximately normal while the other two became marginally normal as each qqnorm plot displays in the figure1 below. After taking log transformation of each of four variables, DSD variable met the normality assumption as Shapiro-Wilk normality test indicates that p-value is 0.092 which is larger than 0.05 indicating that DSD variable has normal distribution.

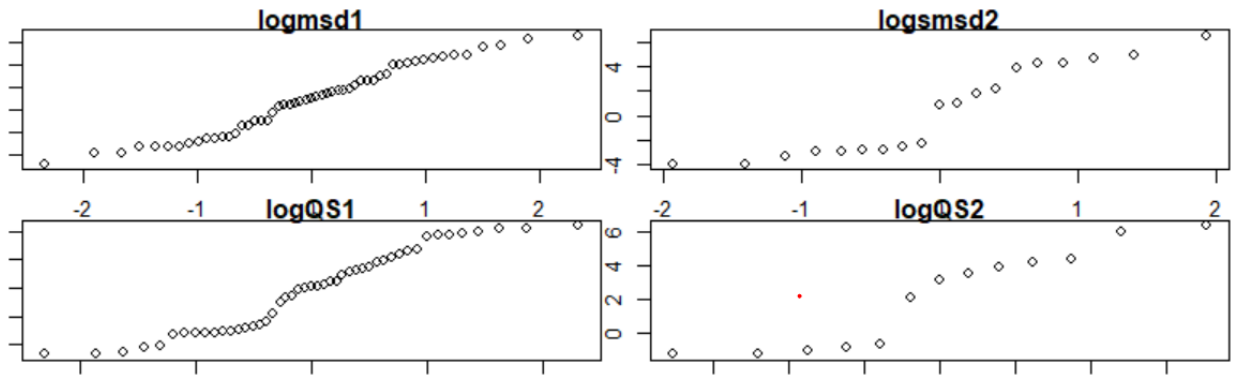


Figure1: q-q plot for each log transformed variables

Using one sample t-test, hypothesis test was done to see if DSD is equal to 0. The null hypothesis and alternative hypothesis are as follows:

H0: The mean difference of squared differences between MSD and QS is equal to 0.

H1: The mean difference of squared differences between MSD and QS is not equal to 0.

- **Cytokine IL-1b**

One Sample t-test for difference of squared differences (DSD) for cytokine IL-1b shows that $t = 2.051$ with $df=8$ and its $p\text{-value}=0.07439$. Since $p\text{-value}$ is larger than 0.05, we cannot reject the null hypothesis at 0.05 significance level. The result indicates that the variance between MSD18 and QS18 is not significantly different from zero. However, 0.7 is not far from 0.05. With only nine data points, difference of squared differences (DSD) has high variance and consequently low precision. Hypothesis test has low power for any reasonable effect size. Taking difference of squared differences reduced the number of data points due to having inordinate number of missing values in MSD18_2 and especially in QS18_2.

- **Cytokine IL-8**

One sample t-test could not be done on difference of squared differences variable for cytokine IL-8 because only two data points are available.

3.2b Paired two sample t-test

The paired two sample T-test was conducted to capture if mean difference between MSD and QS is equal to 0. The null hypothesis and alternative hypothesis are as follows:

H0: The true mean difference between MSD and QS is equal to 0.

H1: The true mean difference between MSD and QS is not equal to 0.

- **Cytokine IL-1b**

For this analysis, instead of taking the difference of squared differences, only MSD18_1 and QS18_1 were compared. Paired t-test for cytokine IL-1b shows that $t=-13.904$ and its $p\text{-value} < 0.0001$. Since $p\text{-value}$ is less than 0.05, we can reject the null hypothesis at 0.05 significance level

indicating that the mean difference between MSD18_1 and QS18_1 is not equal to 0. The mean difference is -1.467, and the 95 percent confidence interval is between -1.680223 and -1.255261. The negative t-test indicates that the mean of QS18_1 is larger than that of MSD18_1.

- **Cytokine IL-8**

Paired t-test for cytokine IL-8 shows that $t = -11.757$ and its $p\text{-value} < 0.0001$. Since $p\text{-value}$ is less than 0.05, we can reject the null hypothesis at 0.05 significance level indicating that the mean difference between MSD18_1 and QS18_1 is not equal to 0. The mean difference is -1.945, and the 95 percent confidence interval is between -2.279059 and -1.612384. The negative t-test indicates that mean of QS18_1 is larger than that of MSD18_1.

In sum, for both IL-1b and IL-8, the result of the paired t-test is consistent in that the mean difference is significant, and mean of QS18_1 score is larger than that of MSD18_1. However, mean of QS18_1 score being larger is not consistent with what the researchers theoretically expected to happen. The rest of the cytokines do not have information about QS18_1 scores. So univariate analyses for comparing MSD18_1 with QS18_1 were done only for cytokine IL-8 and IL-1b.

3.3 Regression analysis

Regression analysis was conducted in two ways. First, the difference of squared differences which represents variance between MSD18 and QS18 was regressed on each of five predictors (Disease Status, CPIS, Ph, CX-ray, Death) to see if the group with any of three lung diseases called case group has higher DSD score than the group without disease called control group, and also to see if any predictor(s) is/are significant in predicting variance between MSD and QS. Second, only MSD scores were regressed on all the predictors in the dataset to see which predictors are significant in predicting cytokine score measured by MSD since QS score is not available for all ten cytokines. In addition, for cytokines measured by both MSD16 and MSD18, each of them was used to see if any of them met the normality of residual assumptions since majority of cytokines measured by MSD16 failed normality of residual assumption. The seven predictors that were used for this analysis: Cxray, CPIS, Ph, Death, Sepsis, ARDS, Pneumonia. Total protein score was not included as a predictor because including that variable reduces the number of observations from 142 to 53 when data sets were merged.

3.3a Results using DSD scores as a dependent variable

- **Cytokine IL-1b**

There are three variables that indicate lung diseases: Pneumonia, ARDS, Sepsis. To compare a group of patients with any of three lung diseases with a group of patients without any of lung disease, Disease Status variable was derived, and 1 was assigned to patient who has any one of lung diseases while 0 was assigned to patient without any one of lung diseases. When difference of squared differences was regressed on Disease status, the estimated regression coefficient of

disease status is -0.08142, this value tells us that the group with diseases (case group) has smaller DSD score than the group without disease (control group). The p-value for t statistics is 0.3268, which is greater than 0.05. This suggests that Disease status alone is not significant in predicting the variance between MSD and QS. When DSD was regressed on each of the rest of predictors (Cxray, CPIS and Ph) one at a time to see if any of them alone is significant in predicting the variance between MSD and QS, none of them were significant at 0.05 significance level. When DSD was regressed on all four predictors as table 2.2 indicates, none of the predictors are found to be significant at 0.05 significance level either. And the adjusted R-squared is only 0.1862. However, when Ph variable was not included in the model, CPIS and Disease status are marginally significant in predicting variance between MSD and QS at 0.05 level as the table 2.1 below shows, and the adjusted R-squared is 0.409, which is better than the model with all four predictors. Coefficients in the tables represents estimated coefficients of each predictor. We wanted to see if the estimated coefficient for Death variable is significant in predicting DSD score but could not estimate it since no death occurrence for the nine observations of DSD scores.

	cxray	CPIS	disease_status
Coefficients	0.018	0.042	-0.155
P_value	0.545	0.065	0.086

Table 2.1 Regression with three predictors

	cxray	CPIS	disease_status	ph
Coefficients	0.001218	0.044675	-0.12937	-0.20326
P_value	0.978761	0.242244	0.284766	0.817907

Table 2.2 Regression with four predictors

For data set IL-8, DSD (Difference of squared differences) could not be regressed on the set of predictors since there are only two data points.

3.3b Results using MSD16, MSD18 scores as dependent variables

- **Cytokine IL-1b**

In order to see which predictors are significant in predicting MSD scores, regression analysis was conducted. Normality of residuals assumption test was first done to check to see if regression analysis is an appropriate method to be used. As the distributions of log transformed MSD16 and MSD18 in figure 2 show that MSD16 is skewed data.

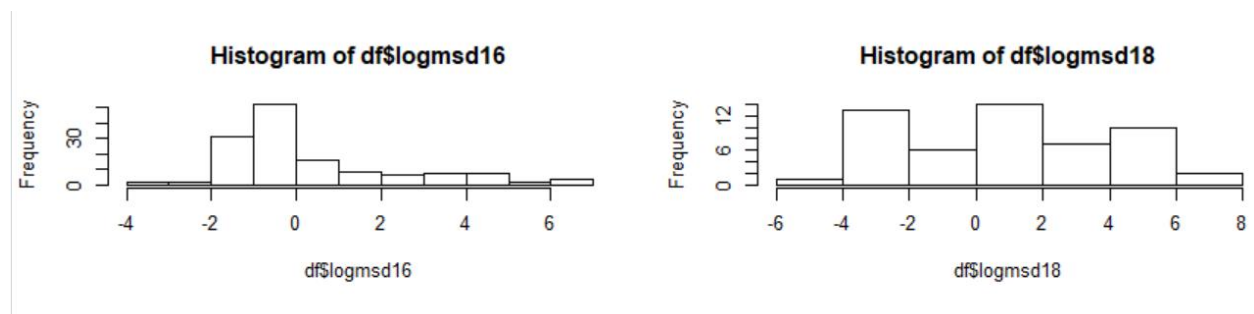


Figure 2. Distributions of log transformed MSD16 and MSD18 scores

Since regression model with the log transformed MSD18 scores met the normality of residual assumption as Shapiro-Wilk normality test indicates that p value is 0.3805, regression analysis is an appropriate method to be used while regression for MSD16 is not appropriate to be used due to not meeting the normality of residual assumption. So only MSD18 score was used for two different sets of predictors. The first set consists of five predictors: CPIS, Ph, Cxray, disease status and Ph. None of the predictors in the first set are significant as each p-value indicates in the table 3.1. The second set consists of seven variables: CPIS, Cxray, Ph, ARDS, Sepsis, Pneumonia and Death. Pneumonia and death are found to be significant in predicting cytokine IL-1b score at 0.05 significance level as shown in the table 3.2. P-values are .0421 for Pneumonia and 0.0470 for Death which indicate that Pneumonia and Death variables are significant in predicting cytokine levels measured by MSD at 0.05 significance levels.

	CPIS	cxray	ph	death	disease_status
Coefficients	-0.03	0.27	3.10	3.12	-2.45
P_value	0.92	0.46	0.69	0.15	0.13

Table 3.1: Estimated coefficients and p-value for the first set

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficients	-0.19332	2.126198	0.220041	-1.85791	-3.03016	-2.4169	4.395745
P_value	0.471405	0.781561	0.532285	0.332073	0.304776	0.04207	0.046974

Table 3.2: Estimated coefficients and p-value for the second set

For the rest of cytokines, normality of residual assumption was not met when log transformed cytokine scores measured by MSD16 was regressed on the set of predictors as appendix 5.1. This indicates that the estimated coefficients are not trustworthy. But it is still reported in the appendix 5.2.

3.4 Mixed effects model analyses

Mixed effects model analysis was conducted in two ways. First, two random effects were used to capture the three sources of variance of MSD scores, QS scores respectively between subject, between samples within subject and between wells within samples. Second, after finding out that variation among wells is negligible compared to total variation as the intraclass correlation indicates in the table 4, one random effect model was conducted to capture which predictors are significant in predicting MSD score.

3.4a Results from mixed effects model analyses

Two random effects model can be represented as follows:

$$y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \varepsilon_{k(ij)},$$

where i represents each subject, j represents each sample, k represents each well, τ_i represents random effect due to subject, $\beta_{j(i)}$ represents nested random effect due to each

sample within a subject and $\varepsilon_{k(ij)}$ represents replication variability of cytokine scores due to wells.

Wells_error_var in the table 3.4 below indicates variance among wells used by both instruments. As shown in the table below, intra-class correlation coefficient(ICC) was calculated for each of three sources of variances: variance due to subject, variance due to samples within each subject and variance due to wells within each sample.

ICC is defined as $\frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2}$ where b represents between and w represents within.

- **Cytokine IL-1B**

For MSD18, ICC for subject is 0.4046; ICC for samples is 0.5835 and the wells_error_var is 0.0118, which represents replication variance due to wells. For QS18, the wells_error_var is 0.00138, which is smaller than that of MSD18 as shown in the table below. The ICC scores for both MSD18 and QS18 indicate that the variance between wells within samples for both MSD18 and QS18 is negligible. This further indicates that the both MSD and QS instruments were working correctly as the client wished. In addition, this also indicates that it would not be necessary to use two random effects. In other words, it is appropriate to use only one random effect model with subject as a random effect.

Three sources of variations for MSD 18 scores						
Subjects	Samples	Error	Total	ICC_Subjects	ICC_Samples	wells_error_var
1.56251	2.25342	0.045878	3.86181	0.40461	0.58351	0.01188
Three sources variations for QS 18 scores						
Subjects	Samples	Error	Total	ICC_Subjects	ICC_Samples	wells_error_var
0.32039	2.30413	0.003633	2.62815	0.12191	0.87671	0.00138

Table 3.4 Comparing three sources of variations of MSD18 and those of QS18 in cytokine IL-1b.

This means that this model allows each subject to have a different baseline average. The model can be represented as follows:

Level-1 model: $Y_{ij} = \beta_{0j} + X_{ij}^T \beta + \varepsilon_{ij}$ where Y_{ij} represents MSD score for each sample and β_{0j} is each subject's mean and ε_{ij} is the sample residual.

Level-2 model: $\beta_{0j} = \gamma_{00} + \mu_{0j}$ where γ_{00} is the grand mean and μ_{0j} is a random effect representing subject's departure from the overall intercept.

We can combine these two models into a reduced form model by substitution

Reduced form : $Y_{ij} = \gamma_{00} + \mu_{0j} + \varepsilon_{ij}$

Using subject Id as a random effect, mixed effect model analysis on IL-1b is used to see which predictor(s) is/are significant in predicting cytokine score measured by MSD16, only ARDS is marginally significant as the highlighted value in the table 3.5 below shows. When MSD18 was regressed on the same set of predictors, Sepsis is statistically significant at 0.05 significance level as the highlighted value in the second Table 3.6 below shows. Sepsis is significant in predicting three other cytokine scores measured by MSD16. They are cytokine IL-10, IL-12p70 and IL-2 as appendix 5.3 shows, and the results of mixed effect model for other data sets are also shown in the appendix 5.3.

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficients	-0.193	2.126	0.220	-1.858	-3.030	-2.417	4.396
P_value	0.559	0.613	0.715	0.444	0.069	0.310	0.434

Table 3.5 -Mixed effect model using MSD16 as response variable

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	-0.388	-0.577	-1.675	0.220	-5.608	6.321	3.807
P_value	0.171	0.123	0.401	0.958	0.005	0.423	0.180

Table 3.6- Mixed effect model using MSD18 as response variable

3.5 Principal Component Analysis

In order to do PCA, two subsets of data were created. One consists of observations with repeated measures of MSD16n score for all five consecutive days while the other consists of observations with repeated measures of MSD16 for the first three consecutive days. There are five such data sets. However only, IL-4 and Tnfa data sets have information on all disease variables while Tnfa does not have information on death variable. From the cumulative proportion of PC1 and PC2, we identified which variables are driving variables. In addition, PCA scatterplots were used as a visualization method to show how the data objects are related to each other and to see if there are clusters. The tables 3.7 and 3.8 displays PCs for two subsets of TNFA data, and PCs for two subsets of IL-4 are shown appendix 5.4. PC1 and PC2 account for cumulative 51% of the covariate variance on average in five consecutive days' data while they account for cumulative 50% of the covariate variance on average in three consecutive days' data. This indicates not much difference in terms of cumulative variance accounted for by two PCs between data set for five days and the data set for three 3 days for both TNFA and IL-4.

	Tnfa PCs for 5 days					
	PC1	PC2	PC3	PC4	PC5	PC6
CPIS	0.54	0.23	0.11	0.56	0.52	0.24
cxray	0.27	-0.59	0.40	-0.17	-0.20	0.59
pneumonia	-0.60	0.40	0.09	-0.06	0.19	0.66
ARDS	-0.08	0.18	0.88	-0.17	0.18	-0.36
sepsis	0.43	0.29	-0.19	-0.78	0.27	0.09
ph	-0.29	-0.57	-0.13	-0.10	0.74	-0.12

Table 3.7: PCA results for Tnfa cytokine for 5 days

	Tnfa PCs for 3 days					
	PC1	PC2	PC3	PC4	PC5	PC6
CPIS	-0.08	0.60	-0.55	0.19	-0.41	-0.35
cxray	-0.10	0.64	0.33	0.42	0.37	0.40
pneumonia	0.27	-0.41	-0.27	0.80	-0.09	0.20
ARDS	-0.58	-0.19	0.25	0.37	0.19	-0.62
sepsis	-0.61	-0.11	0.13	0.00	-0.61	0.47
ph	0.45	0.10	0.66	0.14	-0.52	-0.26

Table 3.8: PCA results for Tnfa cytokine for 3 days

Interpretation of the principal components is based on finding which variables are most strongly correlated with each component. Here a correlation above 0.5 is deemed important. For the data set for three days, the first principal component is strongly correlated with CPIS and Pneumonia while the second component is strongly correlated with Cxray and Ph as the table 3.7 shows. For five days, the first principal component is strongly correlated with ARDS and Sepsis while the second principal component is strongly correlated with Cxray and CPIS as the highlighted values in table 3.8 displays. In addition, The figure 3.5 shows PCA scatter plot with PC1 on the x-axis and PC2 on the y-axis. In this plot, the values of pneumonia and ARDS diseases are shown in combinations of colors and shapes. One on the left is for the subset with three days of MSD scores for TNFA cytokine data while one on the right is for the subset with three days of MSD scores for TNFA. The red circles represent patients without pneumonia or ARDS while green circles indicate patients with pneumonia but without ARDS. The red triangles indicate patients without pneumonia but with ARDS while green triangle indicate patients with pneumonia and ARDS. PC1 and PC2 separate patients according to disease variables as the colors and shapes indicate. However, each cluster is not clearly separated from one another in either in three days' data or in five days' data even though five days' data appear to be better in separating each group of patients. PCA scatter plot on disease variables for IL-4 cytokine has similar pattern in terms of five day's data being better in separating each cluster and in terms of disease variables' being driving variables in PC1 and PC2. PC rotation results and PC scatter plot for disease variables for IL-4 are shown in the appendix 5.4

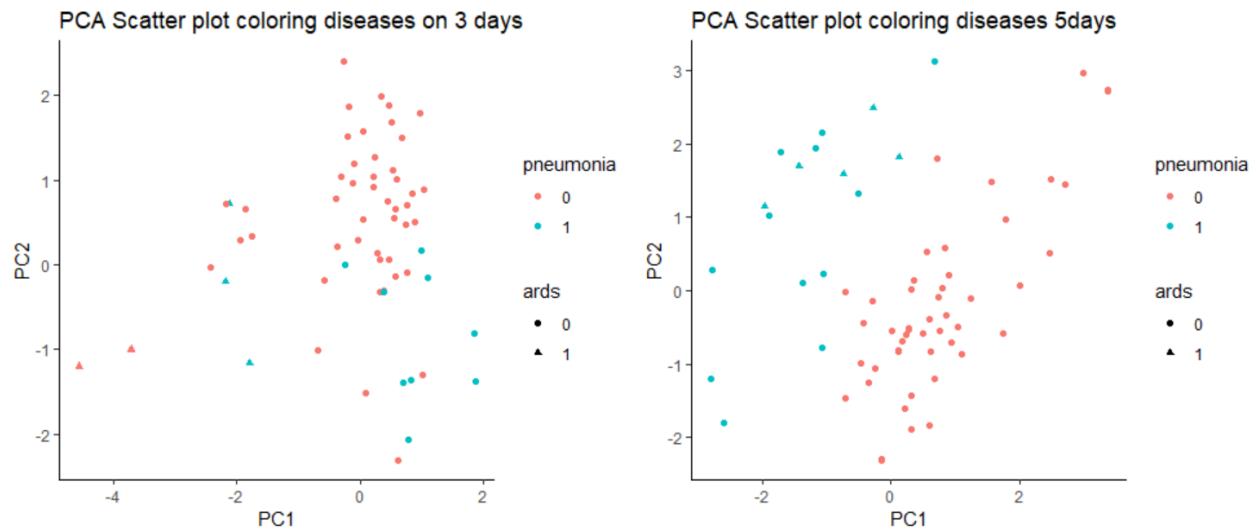


Figure 3.5 PCA scatter plot for cytokine TNFA: one on the left for three consecutive days' subset of data, one on the right for five consecutive days' subset of data

While PCA scatter plots on Death variable could not be done for TNFA due to no such observation being available, IL-4 data set has such observations as shown in the figure 3.6. Death variable is indicated by two colors. PC1 and PC2 in the data set for five days appear to be better than the data set for three days in term of separating the cluster that consists of patients with death from the cluster that consists of patients without death even though PC1 and PC2 do not clearly separate one cluster from the other in either data sets.

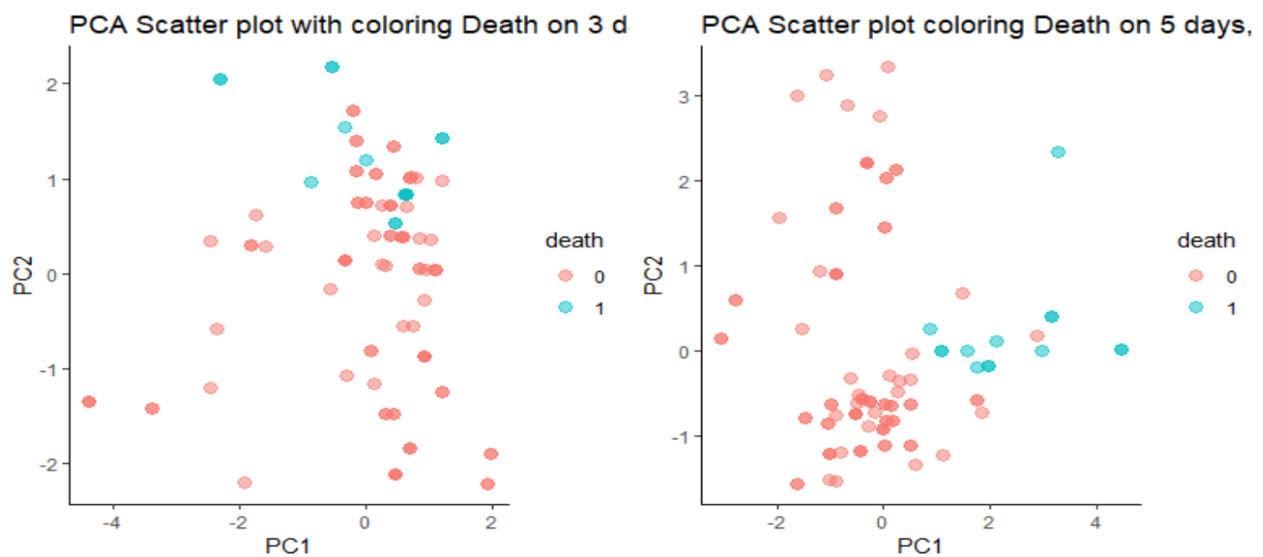


Figure 3. 6 PCA scatter plots for subset of IL-4 data with 3 consecutive days on the left and 5 consecutive days on the right with color on Death variable.

3.6 Logistic regression

Logistic regression was conducted to interpret coefficient as correlation between a hidden continuous and the current continuous variable where hidden continuous variable is each disease variable which is binary variable, and the current continuous variable is each cytokine score measured by MSD16. For each disease, logistic regression was run using cytokine score measured by MSD16 as a predictor and a disease variable as a response variable one at a time to obtain the estimated coefficient. However, coefficients of cytokine for each disease variable were estimated for only three cytokines (IL-1b, IL-6, IL-13) since fitted values of the rest seven cytokines were not feasible due to unboundedness of Maximum likelihood estimate. Hypothesis test was done for coefficient.

Hypothesis testing for coefficient (β) : $H_0: \beta = 0$ vs. $H_1: \beta \neq 0$

As the table 3.9 displays, the estimated coefficient of IL-6 cytokine on ARDS disease is marginally significant since p-value is 0.05. The coefficient of IL-6 cytokine on pneumonia is -0.22574 and odds ratio is $\exp(-0.22574) = 0.7979255$. This means that as one unit increases in MSD16, odds ratio of sepsis disease decreases by around 20%. Since p-value is 0.006 which is smaller than 0.05, we have sufficient evidence to reject the null hypothesis indicating that the effect of IL-6 cytokine on pneumonia is significant at 0.05 significance level. This further implies that there is sufficient evidence to say that correlation between cytokine IL-6 and pneumonia is significantly different from zero.

logistic regression: IL-6			
	ARDS	sepsis	pneumonia
Estimate	-0.31202	-0.14238	-0.22574
Pr(> z)	0.051691	0.161745	0.0064013

Table 3.9: Three separately fitted Estimated coefficient (β)

As table 3.10 shows, the coefficient of IL-13 cytokine on sepsis disease is -0.77721, and odds ratio is $\exp(-0.77721) = 0.4596867$. This means that as one unit increases in MSD16, odds ratio of sepsis disease decreases by around 54%. Since p-value=0.008 is smaller than 0.05, we can reject the null hypothesis indicating that the effect of cytokine IL-13 on sepsis is significant at 0.05 significance level. This further indicates that correlation between cytokine IL-13 and sepsis is significantly different from zero. However, there is no sufficient evidence to show that correlation between IL-13 cytokine and ARDS, pneumonia respectively is significantly different from zero.

logistic regression: IL-13			
	ARDS	sepsis	pneumonia
Estimate	-0.50142	-0.77721	0.0289826
Pr(> z)	0.161057	0.008062	0.9015956

Table 3.10: Three separately fitted Estimated coefficient (β)

In sum, there is no clear pattern in terms of correlation between each of three diseases and each of three cytokine scores. While correlations between cytokine IL-1b and each of three diseases

are not significantly different from zero as reported in appendix 5.5, correlation between cytokine IL-6 and pneumonia and correlation between cytokine IL-13 and sepsis are significantly different from zero.

3.7 Correlation analysis

Since CPIS and Cxray are ordinal variables, Kendall rank based correlation was used to see if correlation between each of CPIS, Cxray and Ph and each cytokine scores measured by MSD16 score is significantly different from zero. Hypothesis test was also conducted.

Hypothesis testing for population correlation coefficient: $H_0: \rho = 0$ vs. $H_A: \rho \neq 0$

If any of the correlation is significantly different from zero, the matrix below is supposed to display one star (★) for a significance at 0.05 level, two stars (★★) for a significance at 0.01 level and three stars (★★★) for a significance at 0.001 level. Each cell in the last column indicates the correlation between each of CPIS, Cxray and Ph respectively and a cytokine score measured by MSD16. No cell in the last column of the matrix for each cytokine displays a star as the figure 3.7 shows. This indicates that there is insufficient evidence to conclude that correlation between each of CPIS, Cxray and Ph respectively and each cytokine measured by MSD16 is significantly different from zero. In sum, across 10 cytokines, no correlation between each of these three predictors (CPIS, Cxray and Ph, respectively and each cytokine score is significantly different from zero even though one common pattern across all cytokines, correlations between each of CPIS, Cxray and Ph and a cytokine are negative. The rest of correlation matrices are shown in appendix 5.6.

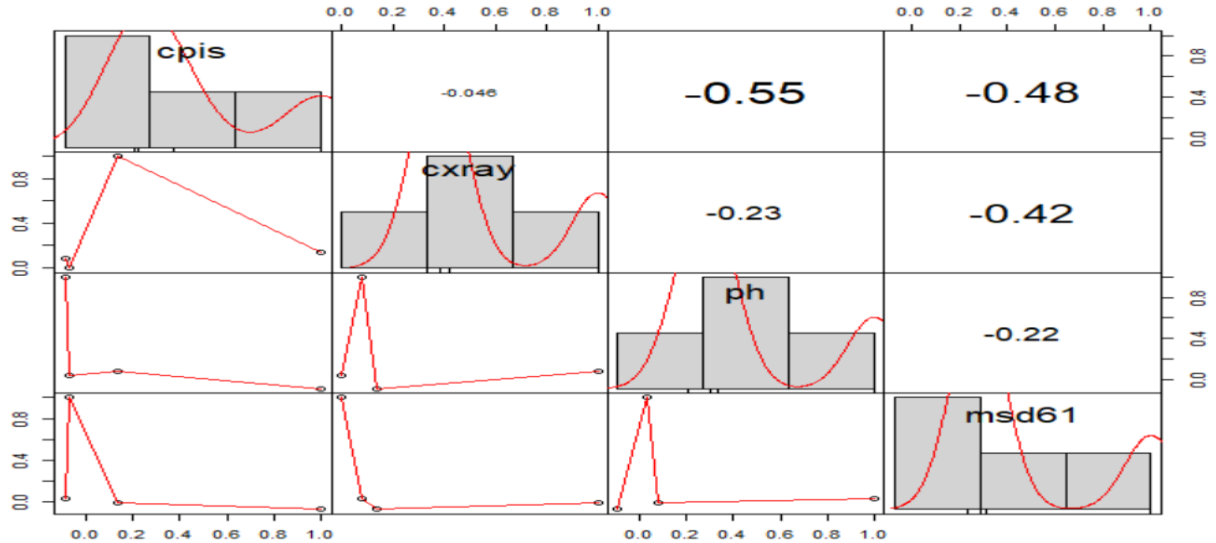


Figure 3.7: correlation between each of three predictors and cytokine IL-1b

4. Summary

For both IL-1B and IL-8, mean of QS18 score turned out to be larger than that of MSD18 score as the paired two sample test indicates. From two random effects model, we captured that QS18 has smaller variation among wells than MSD18. Combining these two results indicates that QS appears to perform better in terms of detecting cytokines with larger mean and smaller variation. The regression analysis on Disease status variable indicates that patients with any of lung disease has smaller cytokine level than patients without any of lung disease. This might be due to the fact that patients with lung disease have difficulty breathing. The regression analysis of MSD scores and mixed effect model analysis results show that disease variables and death are significant in predicting cytokine levels measured by MSD in 2018 for IL-1b cytokine while the result of regression model is not trustworthy for other cytokines since other nine cytokine data sets did not meet the normality of residuals assumption. PCA analysis results from two data sets indicate that on average PC1 and PC2 account for 53 % of variance in the data sets. Disease variables are driving variables in PC1 while CPIS, Cxray and Ph are driving variables in PC2 in both data sets. Five day's data appear to be better in terms of separating each group of patients. The results of logistic regression show that correlations between each of cytokine and each of disease variables vary depending on individual cytokine in terms of whether correlations are significantly different from zero. However, one clear pattern from correlation analysis was captured to see that correlations between each cytokine and each of Cxray, CPIS, Ph respectively are not significantly different from zero.

5. Appendix

5.1 Assumption diagnostics

Name of cytokine	Shapiro-Wilk normality of residual test from regression model
IL-1b measured by MSD18	W = 0.97287, p-value = 0.3805
IL-8 measured by MSD16	W = 0.9657, p-value = 0.003048
IL-4 measured by MSD16	W = 0.93068, p-value = 5.972e-08
IL-2 measured by MSD16	W = 0.9658, p-value = 0.0499
TNFA measured by MSD16	W = 0.78907, p-value = 4.59e-12
IFNG measured by MSD16	W = 0.69834, p-value = 1.251e-14
IL-10 measured by MSD16	W = 0.82496, p-value = 9.843e-11
IL-13 measured by MSD16	W = 0.82496, p-value = 9.843e-11
IL-12p70 measured by MSD16	W = 0.96409, p-value = 0.002221
IL-6 b measured by MSD18	w = 0.95218, p-value = 0.008785
IL-6 measured by MSD16	w = 0.95728, p-value = 1.44e-05

5.2 Regression Coefficients and P values for each cytokines

Cytokine IL-8

Regression coefficients of three disease variables are not estimable for cytokine IL-8 because no subject has any of the disease. So they are excluded from the analysis. Death variable is marginally significant at 0.05 significance level.

	CPIS	ph	cxray	death
Coefficients	-0.10174	0.622526	0.156834	-1.07875
P_value	0.43597	0.869447	0.440737	0.440737

Results by regressing logMSD16

	CPIS	ph	cxray	death
Coefficients	-0.10174	0.622526	0.156834	-1.07875
P_value	0.43597	0.869447	0.440737	0.086849

Results by regressing logMSD18

Cytokine IL-2

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficient	-0.031	-1.522	0.012	-0.450	0.087	-0.019	-0.244
P_value	0.484	0.256	0.859	0.072	0.821	0.930	0.253

Results by regressing logMSD16

Cytokine IL-10

	CPIS	ph	cxray	death
Coefficients	-0.02835	0.281598	0.015663	-0.60518
P_value	0.693567	0.893632	0.888917	0.888917

Results by regressing logMSD16

Cytokine IL-12p70

	CPIS	ph	cxray	sepsis	ARDS	Pneumonia	death
Coefficients	0.069	-0.567	0.156	-0.678	-0.660	0.023	-0.440
P_value	0.135	0.686	0.036	0.010	0.104	0.916	0.050

Results by regressing logMSD16

Cytokine TNFA

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficients	-0.145	-1.675	0.139	-0.724	-0.705	0.126	-0.768
P_value	0.151	0.579	0.385	0.217	0.417	0.798	0.127

Results by regressing logMSD16

Cytokine IFNG

	CPIS	ph	cxray	death
Coefficients	-0.091	-2.638	0.122	-0.263
P_value	0.052	0.053	0.094	0.094

Results by regressing logMSD16

Values of all disease variables are zeros.

	CPIS	ph	cxray	death
Coefficients	-0.102	0.623	0.157	-1.079
P_value	0.436	0.869	0.441	0.087

Results by regressing logMSD18

Cytokine IL-4

	CPIS	ph	cxray	death
Coefficients	-0.102	0.623	0.157	-1.079
P_value	0.436	0.869	0.441	0.087

logMSD16

Cytokine IL-6

	CPIS	ph	cxray	death
Coefficients	-0.0461	6.463896	0.257052	3.775842
P_value	0.865143	0.400431	0.485587	0.485587

log MSD18 as a Dependent variable

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficients	0.021069	8.17981	-0.12594	-1.91965	-0.14135	-2.03881	2.472089
P_value	0.883123	0.060832	0.514204	0.089234	0.923923	0.001013	0.072711

logMSD16 as a Dependent variable

5.3 Mixed effect Model results

SAS code for two random effects with one nested random effect:

```
proc mixed data=_use_ noclprint;
  class subject_id sample_name;
  model log(msd16) =cpis cxray pneumonia lpc ph sepsis death;
  random intercept/subject=subject_id;
  random intercept/subject=sample_name(subject_id);
  ods output covparms=_cov_16;
run;
```

R-code for a random effect:

```
fit_lme=lme(logmsd16 ~ cpis+ cxray +pneumonia+ph+sepsis+ards+death, data=df,random= ~ 1 |subject_id,
na.action=na.omit)
summary(fit_lme)
```

SAS code for one random effect:

```
proc mixed data=_use_ noclprint;
  class subject_id sample_name;
  model log(msd16)=cpis cxray pneumonia lpc ph sepsis death;
  random intercept/subject=subject_id;
  ods output covparms=_cov_16;
run;
```

Cytokine IL-8

logMSD16

	CPIS	ph	cxray	ARDS	sepsis	pneumonia	death
RC	-0.10174	0.622526	0.156834	NA	NA	NA	-1.07875
P_value	0.43597	0.869447	0.440737				0.440737

Values of disease variables are all zero.

logMSD18

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
RC	-0.12677	9.692429	0.112259	NA	NA	NA	3.350953
P_value	0.663426	0.241604	0.775787				0.775787

Values of disease variables are all zero.

Cytokine IL-2

logMSD16

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	-0.04	0.03	0.04	-0.05	-0.58	-2.30	-0.32
P_value	0.34	0.66	0.88	0.94	0.04	0.08	0.31

Cytokine IL-10

Using logMSD16 as a response variable and subject effect as a random effect, Sepsis is significant at 0.05 significance level in predicting MSD16 score while Ph is marginally significant.

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	-0.040	0.032	0.040	-0.048	-0.582	-2.297	-0.319
P_value	0.341	0.657	0.875	0.935	0.039	0.084	0.311

Cytokine IL-12p70

logMSD16

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	0.043	0.097	0.167	-0.564	-0.603	-1.010	-0.465
P_value	0.312	0.179	0.513	0.339	0.034	0.447	0.144

Cytokine IL-TNFA

logMSD16

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	-0.13	0.15	0.06	-0.51	-0.75	-1.24	-0.75
P_value	0.20	0.37	0.92	0.64	0.23	0.68	0.22

logMSD18

	CPIS	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficients	-0.1292	3.145462	-0.04136	-1.23571	-0.67337	-0.74458	1.808111
P_value	0.418495	0.491273	0.84305	0.278511	0.699372	0.281814	0.163135

Cytokine IL-IFNG

logMSD16

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	-0.13	0.15	0.06	-0.51	-0.75	-1.24	-0.75
P_value	0.20	0.37	0.92	0.64	0.23	0.68	0.22

Cytokine IL-4

logMSD16

	ph	cxray	sepsis	ARDS	pneumonia	death
Coefficients	9.69	0.11	NA	NA	NA	3.35
P_value	0.24	0.78				0.15

Values of disease variables are all zero.

Cytokine IL-6

logMSD16

	CPIS	cxray	pneumonia	ARDS	sepsis	ph	death
Coefficients	-0.127	0.147	0.058	-0.510	-0.754	-1.240	-0.752
P_value	0.203	0.367	0.916	0.635	0.235	0.684	0.221

5.4 PCA results

IL-4 for three days

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
CPIS	-0.151	0.587	-0.022	-0.541	-0.229	-0.410	-0.345
cxray	-0.041	0.373	-0.717	-0.072	0.365	0.109	0.442
pneumonia	0.110	-0.530	0.048	-0.623	0.305	-0.384	0.277
ARDS	-0.573	-0.287	-0.267	0.011	0.402	0.074	-0.592
sepsis	-0.593	-0.044	0.039	0.371	-0.178	-0.567	0.392
ph	0.532	-0.040	-0.297	0.399	0.157	-0.583	-0.321
death	-0.017	0.386	0.568	0.129	0.709	-0.078	0.048

Table 5.4a: pc1 and pc2 accounts for 44% of the variance in the data

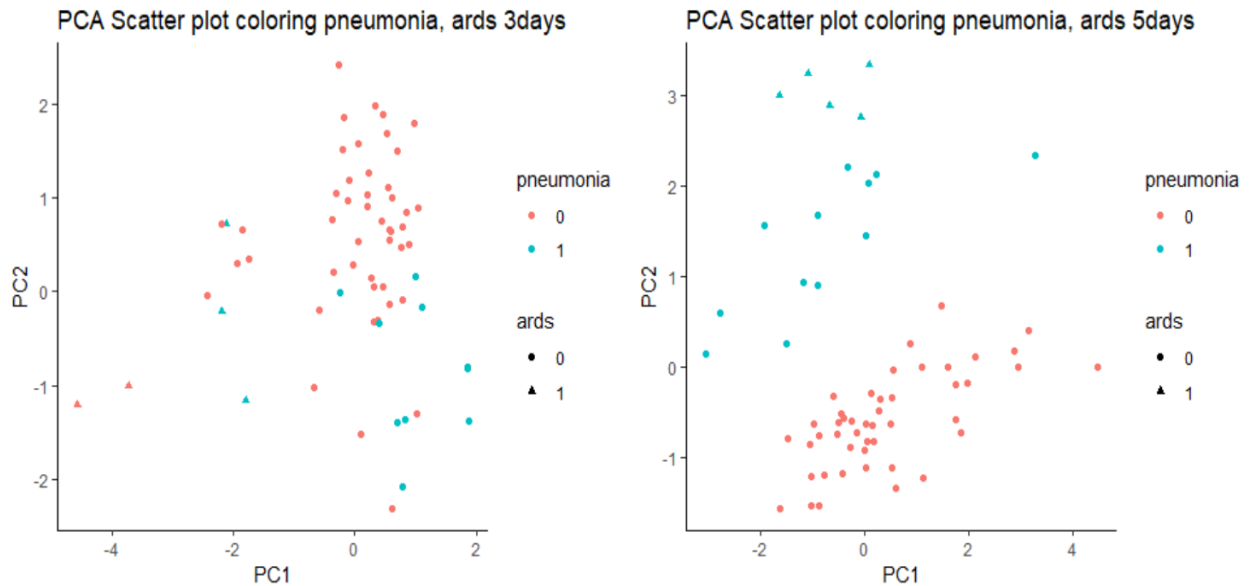
IL-4 for five days

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
CPIS	0.492	-0.063	0.263	-0.164	0.744	0.187	-0.262
cxray	-0.209	-0.442	0.628	-0.077	-0.287	-0.129	-0.513
pneumonia	-0.251	0.697	-0.089	0.112	0.045	0.022	-0.655
ARDS	-0.078	0.456	0.674	-0.018	-0.096	0.374	0.426

sepsis	0.356	-0.104	0.041	0.872	-0.166	0.249	-0.106
ph	-0.520	-0.306	-0.203	-0.001	0.179	0.747	-0.062
death	0.499	0.054	-0.177	-0.440	-0.541	0.434	-0.207

Table 5.4b: pc1 and pc2 accounts for 49% of the variance in the data

IL-4 disease scatter plots



5.5 Logistic Regression

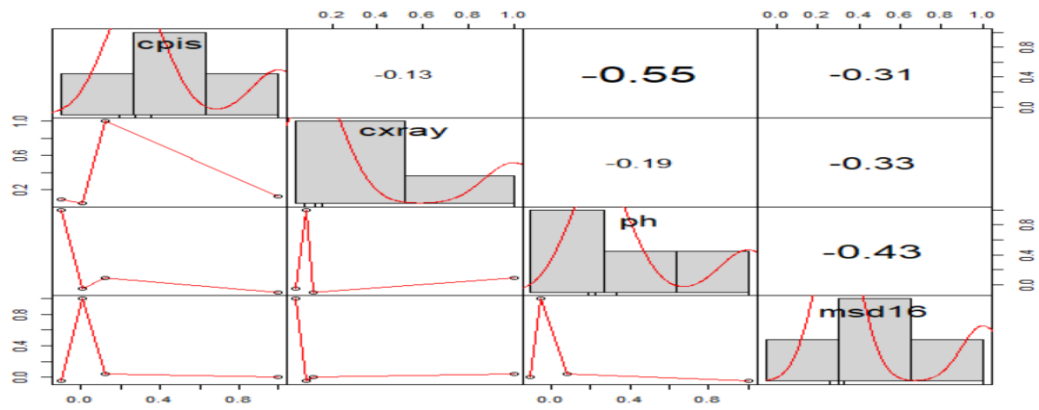
The coefficient of cytokine IL-1b on ARDS disease is 0.215. Odds ratio is $e^{0.25714} = 1.29$. This means that as one unit increases in MSD16, odds ratio of ARDS increases by 29%, and since P-value= 0.408 is larger than 0.05, we fail to reject the null hypothesis at 0.05 significance level. This implies there is no sufficient evidence to say that the effect of cytokine IL-1b on ARDS is significant. This further implies that there is no sufficient evidence to indicate that correlation between ARDS disease and cytokine IL-1b is not zero. Similarly, there is no sufficient evidence to show that correlation between IL-1b cytokine and pneumonia, Sepsis respectively is not significant as p-values in the table 3.9 are shown below.

logistic regression: IL-1b			
	ARDS	sepsis	pneumonia
Estimate	0.21525	0.25714	-0.0808096
Pr(> z)	0.40854	0.17668	0.50120139

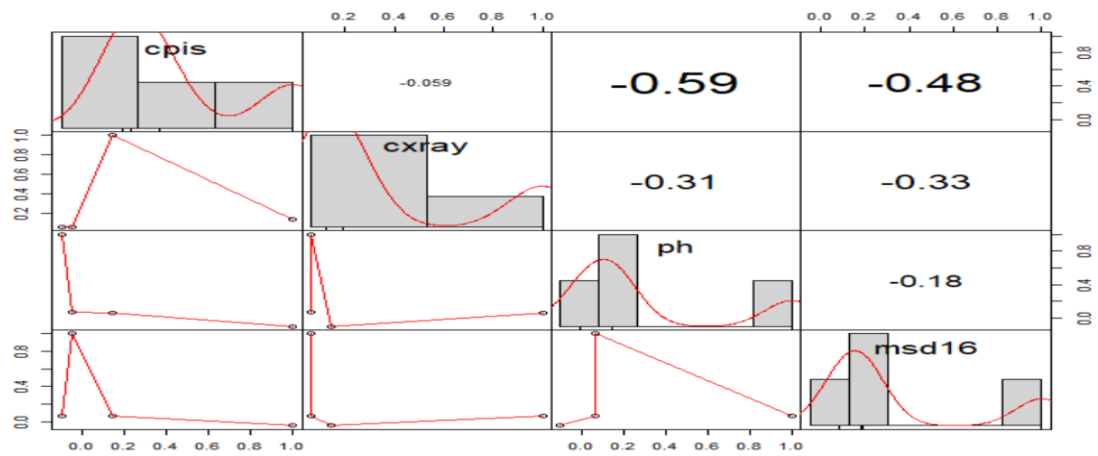
Table 5.5: Three separately fitted Estimated coefficient (β)

5.6 Correlation matrices

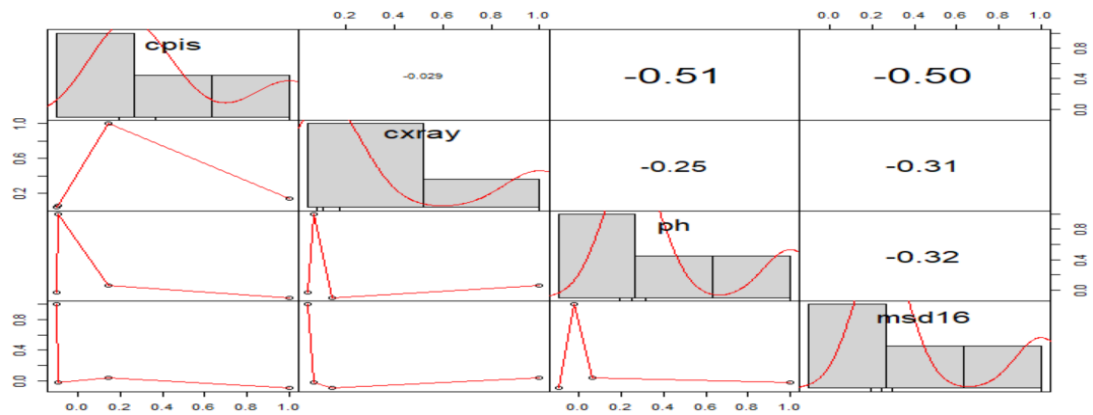
IL-6



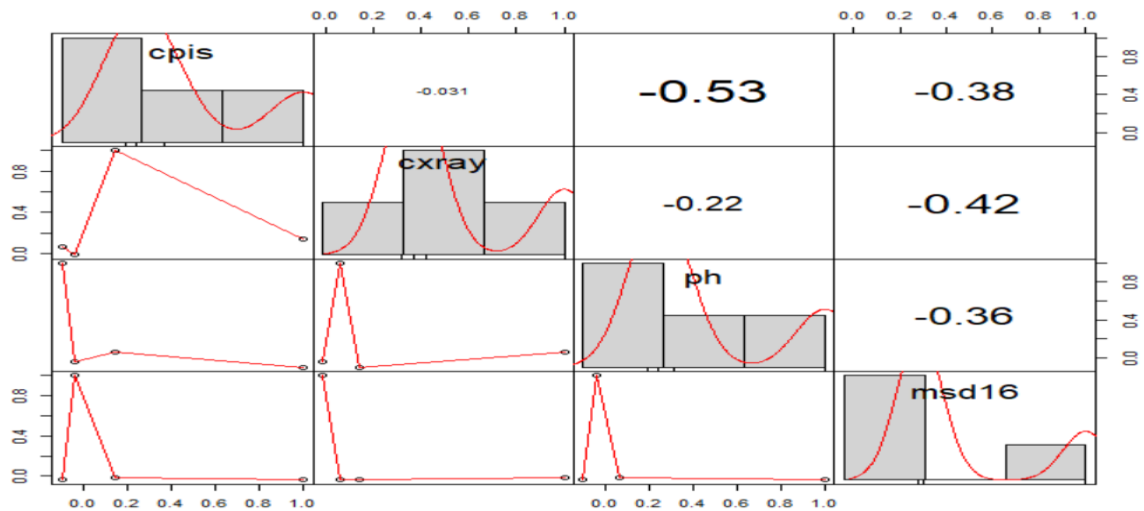
IL-8



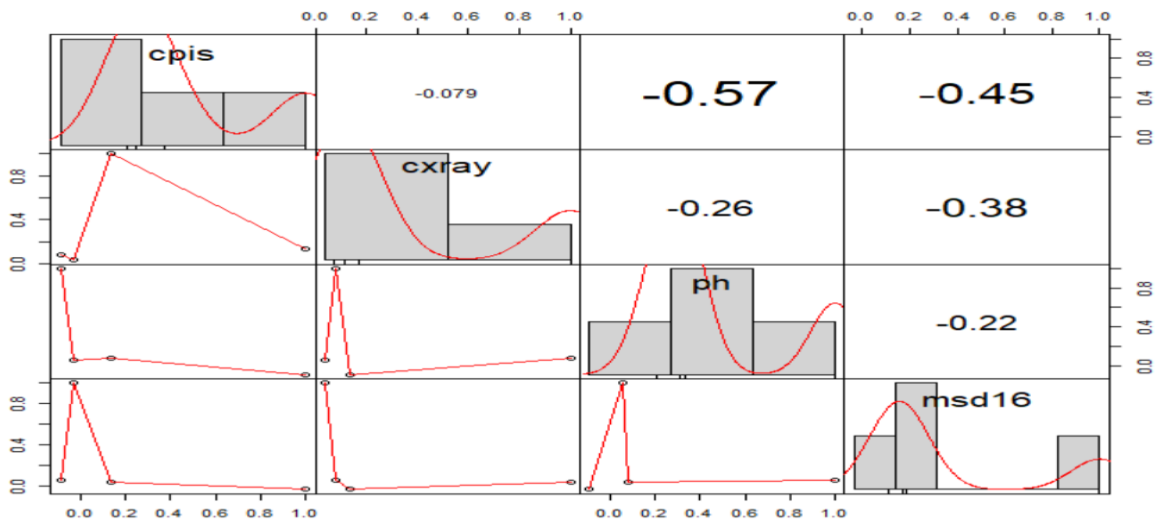
Tnfa



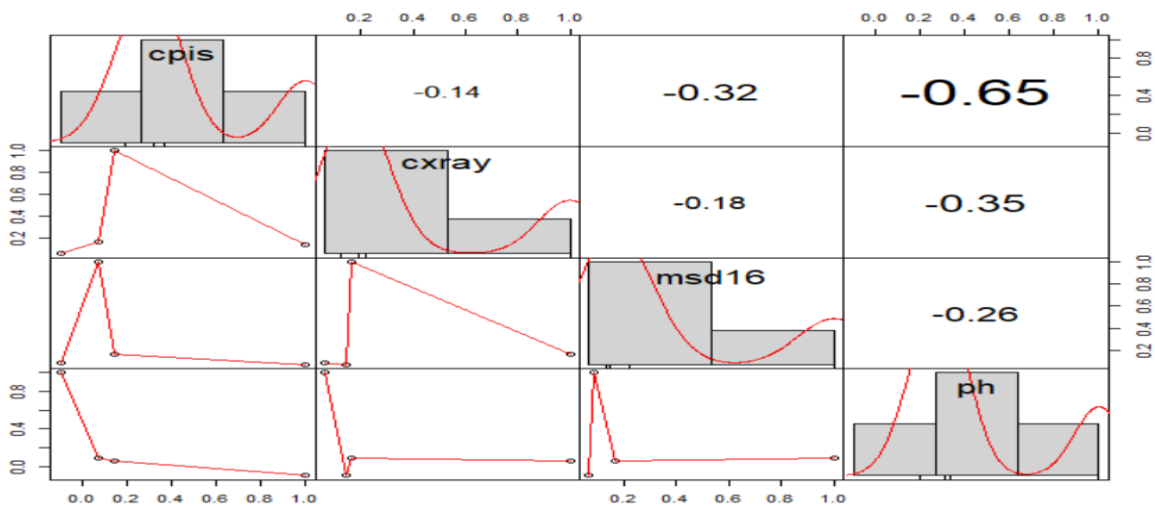
IL-2



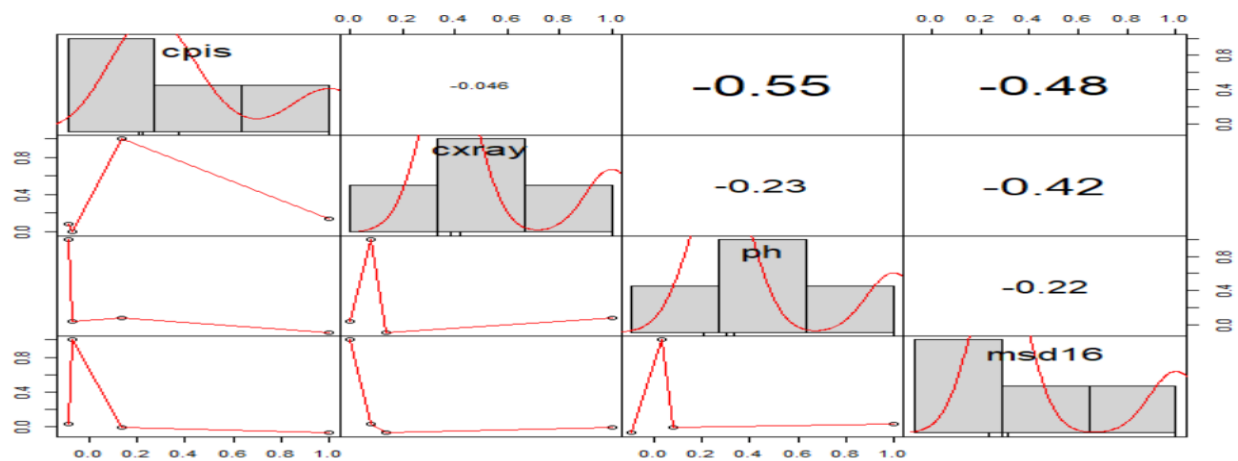
IL-10



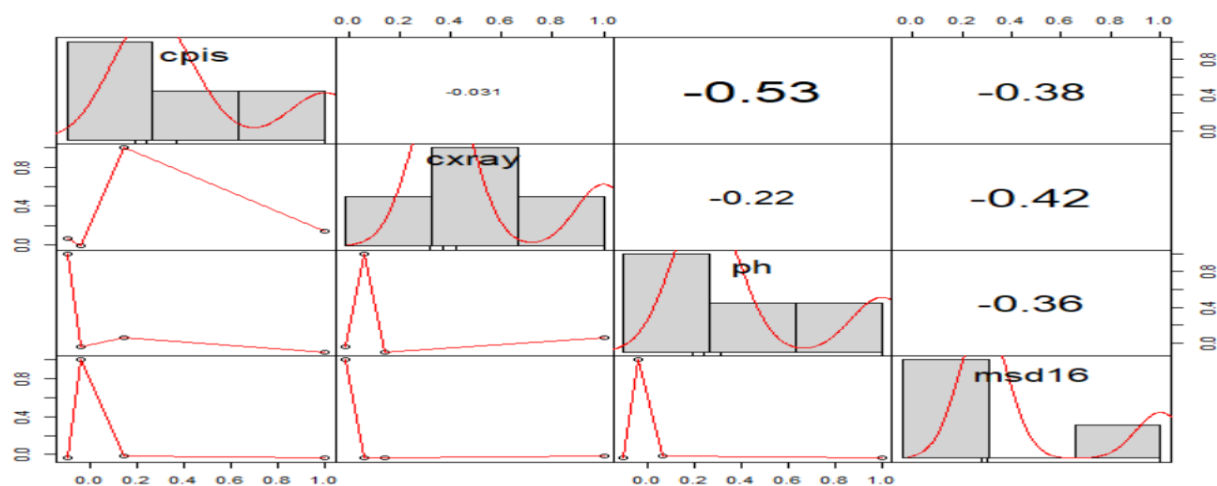
IL-13



IL-4



IL-12p70



IFNG

