**Introduction**

Estimations of cancer expenditure project that on the year 2020 medical spending on cancer can reach over 200 billion dollars[1]. Although pediatric cancer is less prevalent than in the adult [2], the economics and social cost of a child with cancer are tremendous. It can impact profoundly on the kid’s health and development, in the family’s psychological health and economics, and in the society by increasing disability-adjusted life years [3, 4]. The most frequent pediatric cancer is acute leukemia, being the lymphoblastic (ALL) form the most common [2]. On the US, every year 2500 to 3500 new cases of ALL are diagnosed in children [5, 6]. According to the needs, NIH has allocated over the past years a significant amount of resources on Cancer investigation [7]. This research produced advances in the therapeutics that has increase the event-free survival times [8], also provided new insights about the mutations related to ALL that were associated with a variety of outcomes and clinical characteristics.

The research output changed the clinical practices, the clinicians started to classify into groups of risk and treat according to their patients’ clinical phenotypes. The clinical characteristics used to group the patients can differ depending on the research group. For example, Berlin-Frankfurt-Münster (BFM) team base their risk groups solely on treatment response criteria, such as the prednisone prophase response, the minimal residual disease (MRD) at the end of induction phase (week five), and the minimal residual disease at the end of consolidation phase (week 12) [9]. The Children’s Oncology Group (COG) propose to stratify children with ALL according to a subset of prognostic factors like age (aged 1 to <10 years), white blood cell count at diagnosis (<50,000 cells/µL), MRD at the end of induction phase (day 29). COG also considers that genetic findings are important, for example the presence of intrachromosomal amplification or Extreme hyperploidy (59 to 84 chromosomes) or hypodiploidy (fewer than 45 chromosomes), among other factors [10]. Despite the great efforts in research some of the clinical phenotype groups obtained a projected five-year event-free survival of only 75-80%[11-14], this means that probably we are classifying different types of patients together. This implies that we are providing the same treatment to different types of cancer, thus we over treat in some cases increasing the risk of adverse event and in other patients we under treat lessen the chances to obtain a remission.

The research in the field has shown that JAK mutation in high-risk patients had a gene expression signature similar to BCR-ABL1, the last a common mutation in Philadelphia chromosome-positive ALL [15]. Also, gene expression profiling revealed that a rearrangement of cytokine receptor-like factor 2 (CRLF2) is associated with mutations of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome [16]. Research using gene expression profiling and the correlation with genome-wide DNA copy number abnormalities, has been able to establish novel cluster groups that may serve as new targets for diagnosis, risk classification, and therapy [17]. The use of deep whole-exome sequencing has provided insights into the genetics of ALL that could drive the patient to a relapse [18]. Genome-wide DNA copy number and deep whole-exome sequencing are not routinely used in clinical practice, an exception is the gene expression technology. The development of novel methodologies, using the available gene expression technology, to further classify high risk patient with ALL is required.

This project aims to reproduce the proposed by Kang et al [8] methodology to analyze gene expression data. We will develop a COX-regression model based on the principal component analysis (PCA) of the gene expression’s COX-score. Our analysis is limited to open source software. The study findings will be compared against the findings of Kang’s group [8] each step of the process.

**Previous work**

Since Acute lymphoblastic leukemia (ALL) is the most common form of pediatric cancer, it is crucial to classify children with high-risk ALL into different risk groups and provide them with the corresponding treatment[2, 11]. A recent work has shown that the gene expression classifier and flow cytometric measures of minimal residual disease (MRD; P = 0.00Q) each provided independent prognostic information[8, 11]. The combination of these two classifiers improves the risk classification. Methods that were used were supervised learning algorithms and cross­validation techniques. These methods were applied to build a 42­probe­set (38­gene) expression classifier predictive of RFS for 207 uniformly treated children with high-risk ALL[5, 11]. To test the predictive power of gene expression classifier for RFS relative to flow cytometric measures of MRD and to other clinical and genetic variables, they applied a multivariate proportional Cox hazards regression analysis. They used diagonal linear discriminant analysis to build a prediction model between gene expression classifier and end­induction MRD. To evaluate the model, they applied the likelihood­ratio test (LRT) score and the prediction error rate[2, 5].

**Improvements**

By looking at the variables in our dataset, both clinical data and Affy-array data, we could try to find out which variables are the ones with the most significant classification power of high-risk ALL. By including clinical characteristics based on demographics, such as age, race, gender, and ethnicity; clinical data, central nervous system or testicular involvement; laboratory data as white cell count at the time of the diagnosis we are making a prediction model more dynamic and robust. We then would couple with these variables with the Affy-array data to further enhance the prediction power of high-risk ALL. Having a multifaceted prediction model would help us better classify high-risk ALL children, and improve current treatment time. With the current treatment of ALL, time is the key factor in survival rate. If the leukemia is caught at an early stage the survival rate of this high-risk ALL group improves dramatically. For this main reason, we think that this prediction model will save the lives of this high-risk group. We are still looking at our data to see which variables are the best performers. Once we have found which variables are the best performers from our data, we will incorporate them in our prediction model.

**Methodology**

Affy-Array Data:

We obtained the CEL files from the ftp site of TARGET ALL phase 1 project, then we read each file using R affy package and then to normalize the data, we apply a robust multi-array average (RMA) [19] over probes on all the patients. We filter out the probes with features exhibiting little variation, or consistently low signal across the samples [20] over the normalized expression set. The normalization and the filtering process differs from Kang et at [8] methodology, they processed the CELL files using the commercial software Affymetrix GeneChip® Operating Software 1.4.0 Statistical Algorithm package; they filtered out the probe set that were present in less than 50% of the samples. Then we downloaded the clinical data set from the ftp site of TARGET ALL phase 1 project and join it with the filtered expression set. The probe annotations where obtained from the hgy133plus2.db using the annotate package [21].

Statistical Analysis:

We mainly follow the Kang et al. paper[8] and use supervised PCA [22] to detect genes highly associated with survival rate, also classify the risk groups based on their genes instead of pretreatment clinical data (for example: age, ethnicity).

**Part I:** Building prediction model and Cox-regression model

Step 1. Calculate the cox-score (see algorithm next page) for the gene record it as and then we rank the genes by ordering . The bigger the value of the highest association with the survival rate.

Step 2. is a threshold, and we only consider the genes that has > .

Suppose is given, there are genes satisfy > , for the patient, the standardized gene expression level is ()

(1) Principal component analysis is performed on the standardized expression values of the remaining genes. Principal component analysis is a popular approach of dimension reduction and it helps produce a smaller number of linear combinations on variables so that the reduced variables account for and explain most the variance in correlation matrix pattern. Its goal is to extract the important information from the data and to represent the data as a set of new orthogonal variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.

(2) For this data set, we take the first principal component of PCA (most relevant components), which gives loading value of selected genes , then we can get the PCA score for the patient as a linear combination:

(1)

Denote the predicted PCA score for 207 patients as .

(3) Cox proportional hazard model assumes that the instantaneous event rate at time is of the following form:

When covariate is at different level, say , the ratio of hazard rate is proportional to the difference of the covariate level:

)

We assume the hazard rate for the relapse of leukemia follows the Cox model, the covariate here is the PCA score (see equation (1)), and the hazard rate is . Since PCA score is a linear combination of the highly associated genes, we evaluate the genes related to the hazard rate of leukemia.

Cox-regression can be done with the coxph(surv(time,status)~). The LRT (likelihood ratio test) for this model will be reported as a measure of goodness of fitting.

Step 3: Test the performance of the cox-regression model:

Follow from the Cox model , we fit the coefficient with . For the patient, the linear combination of the genes

(3)

is used as a prediction model.

To exam the power of the prediction, we can predict the PCA score on new samples, let’s say patient , and get . Then cox-regression can be fitted to the survival time and the gene expression level of the new samples. The performance of the cox-regression can be evaluated by LRT. The larger LRT, the better the performance.

**Part II:** Using CV (cross validation) to select threshold

In part I step 2 is under the condition that is given. To select , an easier way might be we just assign a value to , so that it only includes the top, let’s say, 10% or 5% highly expressed genes. However, this kind of assignment is not that convincing, and one possible way to improve it is to use CV to select.

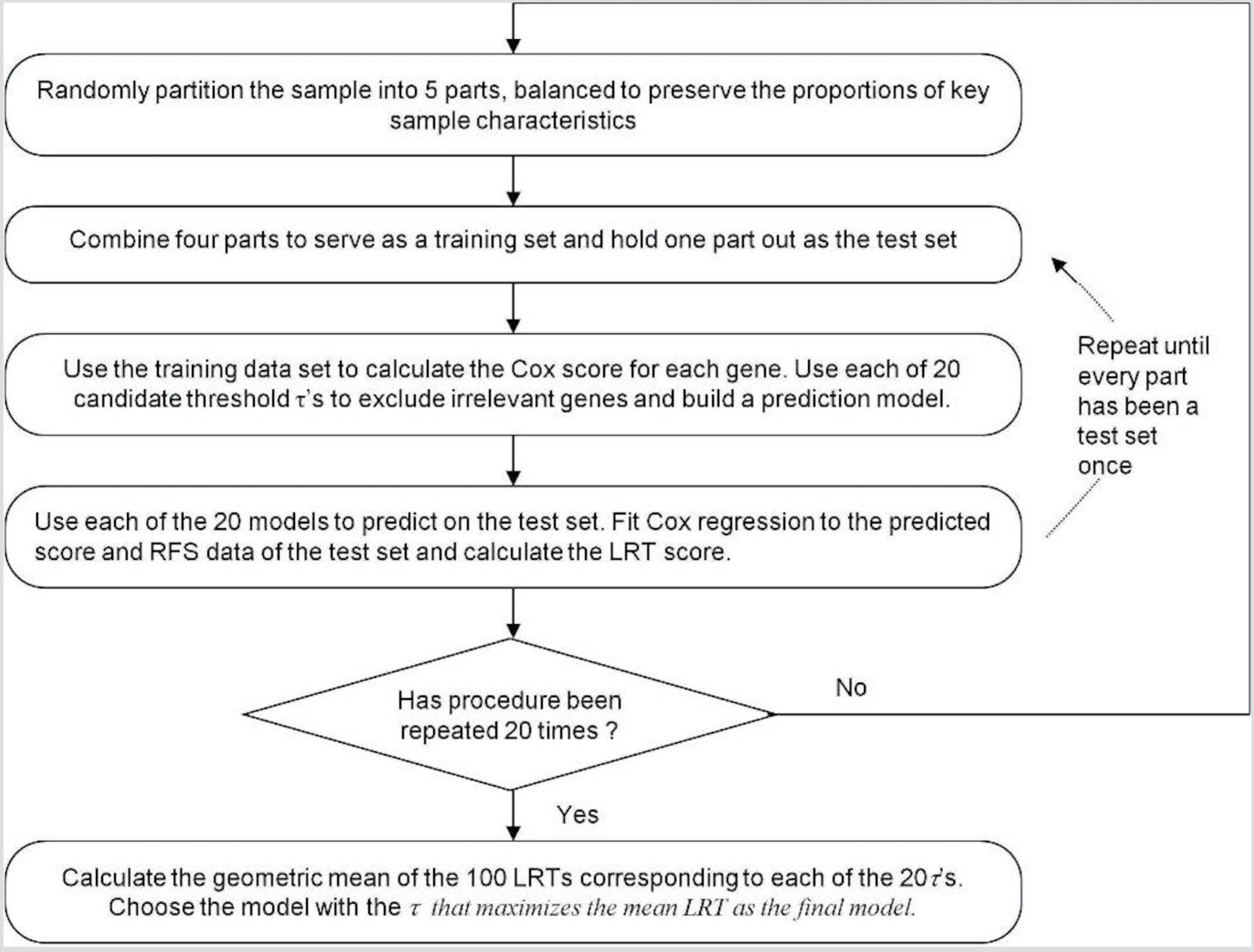
Cross-validation is primarily a way of measuring the predictive performance of a statistical model. One way to measure the predictive ability of a model is to test it on a set of data not used in estimation. In many real problems, we don't have enough data to set aside a large test set and this motivates the CV. The idea for using CV is to avoid over fitting and make model more robust.

The general ideal of k-fold CV is interpreted in the following graph[23].



**Part III** Supplementary[8]:

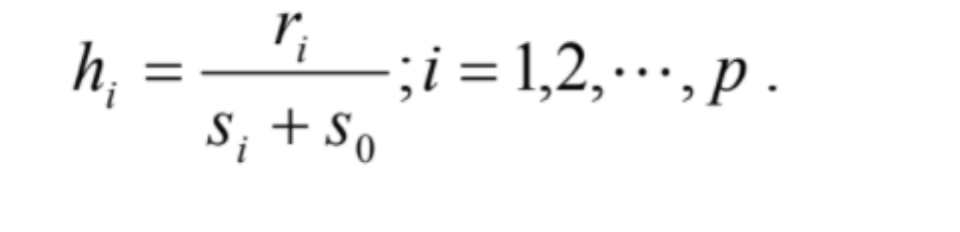
1. To select , an example of 5-fold CV for selecting combined with part I is the following graph:

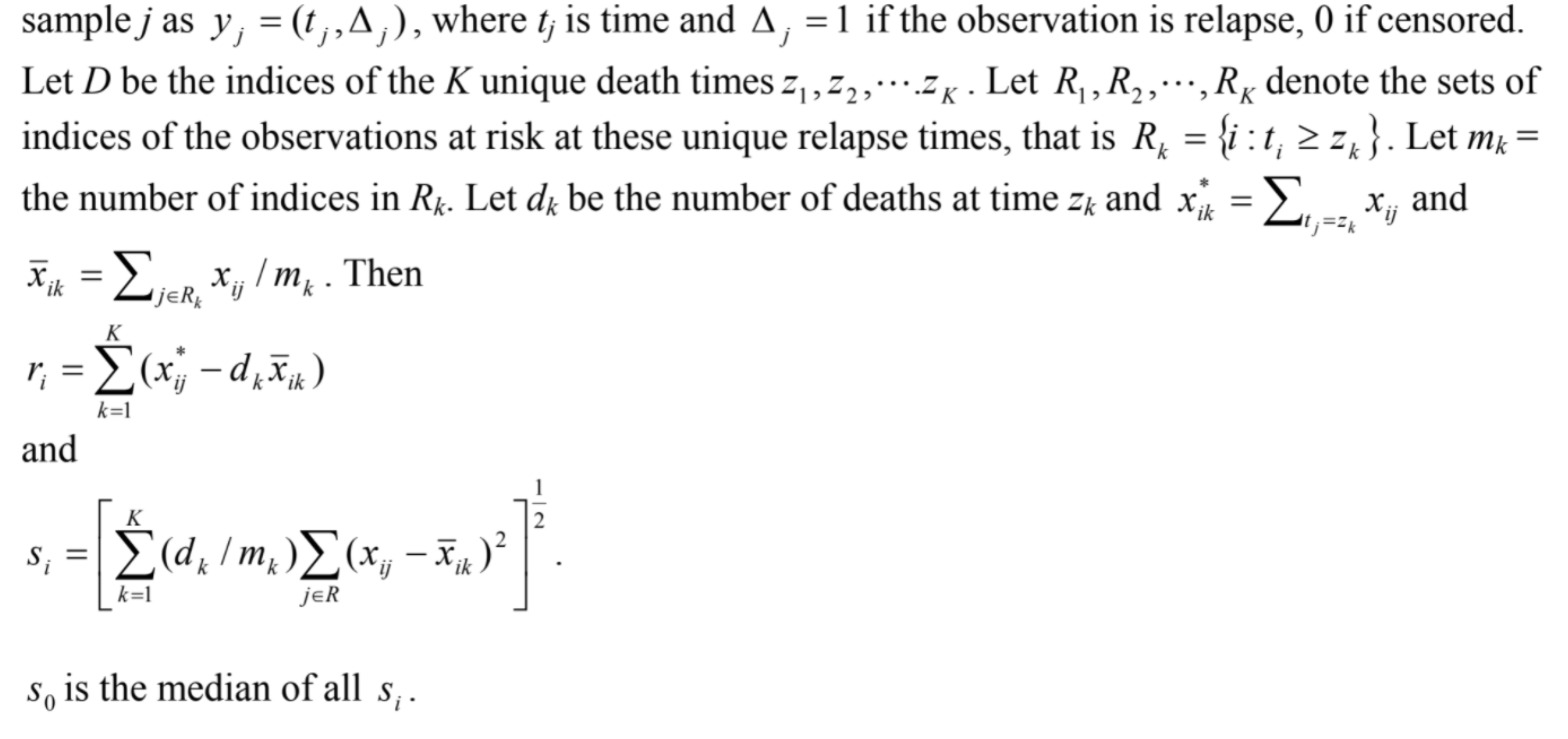


2. Calculating cox-score:

Let denote genes and denote patients.

The cox score for gene is :





**Expected results**

(1) After applying the algorithm in the supplementary part, we will have cox score for each gene.

(2) Based on the cox score of genes, we can set possible candidates threshold.

(3) Randomly partitioning the 207 patients to test set and training set.

(4) Perform CV on the training and test dataset. we need to recalculate the cox-score for each gene based on the training dataset, then select genes based on different threshold, do PCA and cox-regression. Predict the PCA score and perform cox-regression in the test dataset. Each threshold will give us a LRT score. We can select the threshold base on the highest LRT score. (or based on the principle of parsimony, select the second or third highest LRT which corresponding to a set of fewer genes).

(5) The risk of each patient can be predicted by PCA score and further classify the patients on high/low risk group.

(6) Use Kaplan-Meier estimator to plot the survival rate of the high/low risk group, which would be a fine classification of the 207 patients.

**Results**

By reading the 207 downloaded CELL files and after the RMA, we obtained for each patient 54,675 probes (data frame of 54,675 rows and 207 columns). After filtering the probes using the described criteria we ended up with 21,148 probes per patient.

Table1. Comparison of microarrays data processing

|  |  |  |
| --- | --- | --- |
| Step | Kang et al. [8] | Group project |
| Cell Files | 207 | 207 |
| Probes after RMA | 54,675 | 54,675 |
| Probes after filtering | 23,775 | 21,148 |

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