Classification Algorithm for High Dimensional Protein Markers in Time-course Data

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Abstract

Identification of biomarkers is an emerging area in Oncology. In this article, we develop an efficient statistical procedure for classification of protein markers according to their effect on cancer progression. A high-dimensional time-course dataset of protein markers for 80 patients motivates us for developing the model. We obtain the optimal threshold values for markers using Cox proportional hazard model. The optimal threshold value is defined as a level of a marker having maximum impact on cancer progression. The classification was validated by comparing random components using both proportional hazard and accelerated failure time frailty models. The study elucidates the application of two separate joint modeling techniques using auto regressive-type model and mixed effect model for time-course data and proportional hazard model for survival data with proper utilization of Bayesian methodology. Also, a prognostic score has been developed on the basis of few selected genes with application on patients. The complete analysis is performed by R programming code. This study facilitates to identify relevant biomarkers from a set of markers.

Keywords : Classification, Frailty, Joint Modeling, Auto-regressive, Bayesian.

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1 Introduction

Biomarkers are measurable biological characteristics used as indicators of the occurrence and progression of disease. They play a crucial role in identifying disease onset as well as improving drug development process. The detection of cancer at primitive stages is based on the identification of deviation of biological characteristics from the normal status. Thus, potential biomarkers can be identified among specific proteins or peptides which are evaluated higher or lower in cancer patients compared to controls.

Protein molecular biomarkers are particularly popular due to their potential for early detection of disease or providing information about the risk of disease [1]. To measure cancer progression more effectively, it is required to collect observations multiple times and hence, circulating biomarkers are becoming more popular in cancer treatment. Circulating biomarkers identified in biological liquid samples, termed as liquid biopsies, are particularly important in identification of tumor cells [2]. There are several advantages of liquid biopsies over tissue-based profiling; they are minimally invasive, can be sampled repeatedly, collect information efficiently with less sampling bias and allow longitudinal tracking of patient response to therapies [3].

Gene expression data, acquired from microarray experiments, are increasingly used to measure changes in the gene expression over time [4], [5]. The analysis of temporal changes of gene expression is useful to comprehend the complex mechanism of gene and its effect on disease progression. One of the critical problems associated with gene expression data is its high dimensional structure. Single gene expression data or expressions of only a few genes are easy to analyze. However, the analysis becomes difficult when the number of gene expressions is high and it is necessary to accommodate all the genes together in the analysis. Classical statistical tools fail to analyze such high dimensional gene expression data. There are several approaches including gene-by-gene statistical analysis, dimension reduction methods or gene set analysis which acknowledge such kind of data (6, 7, 8). Gene set analysis is an important tool to analyze high-dimensional gene expression data and also considered to be more powerful than gene-by-gene analysis. It can detect a change in expression of a group of genes that are functionally linked. The time-course gene set analysis (TCGSA), which is an extension of gene set analysis, relies on random effects modeling with maximum likelihood estimates. TCGSA is a hypothesis-driven method that takes into account the potential heterogeneity of gene expressions and distinguishes genes with significant temporal variations in expressions. Other methods adapted and found suitable in analysis of genes are namely Gene Set Enrichment Analysis, sigPathway, Significance Analysis of Function and Expression etc [9], [10], [11].
Select genes $g_i$, $i = 1, 2, ..., 500$

time points $t_j$, $j = 1, 2, ..., 5$

gene expression $g_{i,t_j}$,
$i^{th}$ gene for $j^{th}$ time-point

For the first time-point $t_1$

Range at time $t_1$ for $i^{th}$ gene $g_i$: $[a_{i,t_1}, b_{i,t_1}]$

Make two partitions
$[a_{i,t_1}, a_{i,t_1} + s_{i,t_1}]$ and $[a_{i,t_1} + s_{i,t_1}, b_{i,t_1}]$

Classify:
if $g_{i,t_1} \in [a_{i,t_1}, a_{i,t_1} + s_{i,t_1}]$, $D = 0$,
if $g_{i,t_1} \in [a_{i,t_1} + s_{i,t_1}, b_{i,t_1}]$, $D = 1$

Fit CoxPH and find the p-value

Compare p-values of for all $s_{i,t_1}$ for gene $g_i$

Find threshold value and $\beta$ parameter for minimum p-value for time $t_1$

Accept
Reject
Accept

All $\beta$ are +ve (Consistent Gene)
Mixture of +ve and -ve values (Noisy Genes)
All $\beta$ are -ve (Consistent Gene)

Look at the sequences of $\beta$ for all genes

Get the sequence of $\beta$ for all time $t_1$, $t_2$, ..., $t_J$

In oncology research, the effect of gene expression on the occurrence of death or disease progression is a challenging area for research and cultivation. For high-dimensional longitudinal gene expressions data, it is necessary to identify the change in effect of the gene on an event (disease occurrence or death) and clustering genes according to that. For longitudinal gene expression data, it is observed that the measurements are often dependent over previous values, i.e., they are auto-correlated. The baseline expression value portrays a significant role and often the recent expression value is compared to the baseline value to draw the necessary inference [12]. Associating longitudinal gene expressions with the event occurrence
using joint modeling technique carries significant importance in this kind of study. The longitudinal process can be explained using an auto-regressive model or standard mixed effect model. For survival analysis, there are some well-known available methods like Cox proportional hazard model or accelerated failure time model.

Several approaches have been proposed to analyse longitudinal gene expression measurements but only a few address high-dimensional gene expression data. Also there is a clear lack in usage of joint modeling technique in gene expression study. Also as per the knowledge of authors, there are absolutely no literature on use of auto-regressive joint modeling in this area of research which is quite obvious due to the computational difficulty. In this article we present a detailed procedure of joint modeling using both ‘auto-regressive type’ longitudinal model and standard mixed effect joint model. Bayesian methodology is applied in fitting joint model. The data is analysed with proper programming code using R opensource software. R-INLA package is used for survival analysis and joint modeling using bayesian techniques. In section 2, we explain the filtration method of markers using proportional hazard model. In section 3, the clustering method is validated using frailty effect and the protein markers are ranked according to their frailty. In section 4, we analysed longitudinal data using auto-regressive model. In section 5 and 6, the joint modeling technique is described using auto-regressive type longitudinal model and standard mixed effect model respectively. In section 7, some discussions are made on our proposed procedure and future scope of this work.

2 Methodology

2.1 Cox Proportional Hazard Model and Filteration:

Analysis based on duration of survival in relation to occurrence of death is considered as survival analysis. The application of survival analysis has been broadened in a variety of disciplines with the definition of survival duration and event history. It stands apart as an useful tool by accommodating the censoring of event due to non-occurrence. The event of interest is often considered as death which phrases such terminology to this vast area of study. There is a vast literature available on survival analysis which has been broadened by a large number of researchers ([13], [14], [15], [16]). However, very limited literature is devoted towards the development of statistical methods for the analysis of multi-level survival data [17]. Frailty models are also introduced and described to analyse clustered survival data ([16], [18], [19]). The hierarchical linear model (HLM) was introduced as the primary method to analyze multilevel data with continuous variables whereas The hierarchical generalised linear model (HGLM) was introduced to accommodate discrete outcomes. However, very limited resources of multi-level analysis is dedicated to
provide a detailed discussion of multi-level survival analysis with emphasis on applications using softwares [20].

Suppose that there are \( n \) independent subjects (i.e., patients). Let \( T'_i \) be the time to failure for the \( i^{th} \) subject, \( i = (1, ..., n) \) and \( Z_i \) is a \( k \times 1 \) vector of covariates. The failure time is subject to non-informative right censoring \( C_i \). Also we assume that \( \Delta_i = 0 \) indicates the failure time is censored and \( \Delta_i = 1 \) indicates the event observed. So, the failure time \( T_i \) is considered as \( \min(T'_i, C_i) \). Therefore, the observed data consists of \( \{T_i, \Delta_i, Z_i\} \) for \( i = 1, ..., n \). \( T'_i \) and \( C_i \) are independent given \( Z_i \) for each \( i \).

The hazard function for the \( i^{th} \) subject is defined as,

\[
\lambda_i(t|Z_i) = \lim_{h \to 0} \frac{Pr(T_i \in [t, t+h]|Z_i)}/h
\]

For each patient, the survival duration is split into several parts based on their follow-up dates. So for a patient \( i \), the duration is computed only from the last follow-up. So, the 1st duration is computed as 1st follow-up date to 2nd follow-up date, 2nd duration is computed as 2nd follow-up date to 3rd follow-up date and so on. Whenever an event occurs, the duration between last follow-up to the disease occurrence time is considered. Here it is assumed that the occurrence of disease greatly influences the gene expression. So, the value of gene expression at immediate follow-up can be considered as the value of gene expression at the event occurrence time. Hence to calculate time duration, the immediate sampling date post disease occurrence is ignored and the next duration is taken as the time span between 2nd immediate follow-up date and disease occurrence date.

Assume that for a patient \( i \), the duration of survival is \([0, d_i]\) where,

\[
d_i = \begin{cases} 
C_i, & \text{when there is no event occurrence or,} \\
\text{the event happened in between follow-ups.} & \\
T'_i, & \text{when the event occurred after the last follow-up}
\end{cases}
\]

The survival duration of a patient is divided into a few parts based on number of follow-up. We assume that the number of follow-ups for patient \( i \) is \( l_i \) where the maximum value of \( l_i \) is 4. Let the duration between follow-ups are \( t_1, t_2, ..., t_{l_i} \). The gene expressions are assigned according the above defined procedure. The survival durations are assumed to be independent.

To analyse survival data, one of the most widely used parametric method is Cox proportional hazard model (Cox, 1972). For \( i^{th} \) individual with \( j^{th} \) time point, the proportional hazard model fitted for a particular gene can be written in simple terms as,

\[
\lambda_{ij}(t|Z_{ij}) = \lambda_0(t)exp(Z_{ij}\beta)
\]

or,

\[
\log \lambda_{ij}(t|Z_{ij}) = \log \lambda_0(t) + Z_{ij}\beta, \quad j = 1(1)l_i
\]
where $Z_{ij}$ is an indicator function defined as follows. For each gene $g$ at time point $j$, $j = 1(1)4$, the expression values are divided into deciles and we have to find the optimal threshold value of activity of these genes. For a particular $p^{th}$ decile $[p =1(1)9]$, the expression is divided into two parts, $[\min Y_g, Y_{(g,p)}]$ and $[Y_{(g,p)}, \max Y_g]$. 

\[
Z_{ij} = \begin{cases} 
0 & \text{for } Y_{g,ij} < Y_{(g,p)} \\
1 & \text{for } Y_{g,ij} \geq Y_{(g,p)}
\end{cases}
\]

In this way, the proportional hazard models that are fitted for different indicators on time-point $j$ for gene $g$ are,

\[
\log \lambda(t_{ij}) = \log \lambda_0(t_{ij}) + I(Y_{ij} > Y_{(p)})\beta_j, \ p = 1(1)9
\]

For each equation, we obtain a corresponding p-value which demonstrates the significance of the covariate. Lower the p-value, greater is the significance of the covariate on response. Here, the equation with minimum p-value gives highest importance on the indicator which produces the highest significant level of gene expression. For each time point, we obtain the threshold values of gene expression.

Our interest is to classify the genes according to their effect on the disease occurrence. Gene expressions of some particular genes increase with the disease occurrence, while for some gene expressions, the disease affects negatively on expressions. So it is important to take into account the signs of the parameters associated with the indicator of threshold values. For the $k^{th}$ gene at $j$ time-points, the parameters obtained using Cox proportional hazard model are $[\beta_{k1}, \beta_{k2}, ..., \beta_{kj}]$. If $\beta_{kj} > 0$ for all $j$, then the gene is called Progressive Gene and if $\beta_{kj} < 0$ for all $j$, then the gene is called Oppressive Gene. Other than these two type of genes, there are some other genes which do not have a consistent positive or negative association with the disease occurrence over time i.e., $\beta_{kj}$ is neither $> 0$ nor $< 0$ for all $j$. These genes are called Volatile Genes.

### 2.2 Frailty Effect in Gene Expression

The frailty is an important aspect to introduce randomness in survival models. The unobserved heterogeneity that influences the hazard function can be addressed using a random factor in survival modeling. Earlier the frailty model was denoted as a survival regression model (either a Cox proportional hazard model or any other parametric model) that incorporates a random component. Later it is defined as survival model with only random intercept whereas mixed effect model is used to refer a model with multiple random effects \[21\]. Thus, the frailty model is referred as a special case of mixed effect survival models. Earlier, the subject specific random effects were primarily adddressed by frailty models to account the

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unmeasured subject characteristics. These models were subsequently extended to incorporate cluster specific random effects to address within-cluster homogeneity effect in hazard rate. These models are referred as shared frailty model as the frailty is shared among all the subjects in same cluster ([16], [18]).

Cox regression models with mixed effects are popularly used to address shared frailty. Different distributions have been considered for the shared frailty terms including Gamma distribution, Weibull distribution, Log-normal distribution, positive stable frailty distributions etc. ([22], [23], [19]). The gamma and log-normal distributions are most commonly used for frailty terms. The term ‘nested frailty model’ was pioneered by Rondeau et al. to refer survival models with multi-level clustering [24].

In proportional hazard model, the frailty effect can be associated in the following way. Let \( t_{ij} \) be the survival time for the \( j^{th} \) individual in the \( i^{th} \) cluster, \( i = 1(1)n, j = 1(1)m_i \). Keeping similarity with the notations as defined in proportional hazard model, we define \( C_{ij} \) as the censoring time and \( T_{ij} \) as the event time. The failure time \( X_{ij} \) is defined as \( \min(T_{ij}, C_{ij}) \). Also the censoring indicator is defined as \( \Delta_{ij} = I(T_{ij} < C_{ij}) \). Also, we define the no. of observed events as \( d_i = \sum_{j=1}^{m_i} \Delta_{ij} \). The frailty model is defined as,

\[
\lambda_{ij}(t | w_i, Z_{ij}) = \lambda_0(t) exp(Z_{ij} \beta + w_i) \quad (5)
\]

where \( h_0(t) \) and \( \beta \) is same as defined in proportional hazard model. \( w_i \) is the random effect for the \( i^{th} \) cluster. Here, \( w_i \)'s are the actual values of a sample from density \( f_w \). This model can be rewritten as,

\[
\lambda_{ij}(t) = \lambda_0(t) u_i exp(Z_{ij} \beta) \quad (6)
\]

where \( u_i = exp(w_i) \) is the frailty for the \( i^{th} \) cluster.

Another way of adressing frailty in survival model is using accelerated failure time (AFT) model. With usual notations as mentioned before, the AFT frailty model is defined as,

\[
S_{Z_{ij}}(t) = S_0(exp(-Z_{ij} \beta - w_i)) \quad (7)
\]

The model can be written as,

\[
log(T_{ij}) = \mu + Z_{ij} \beta + u_i + \sigma \epsilon_{ij} \quad (8)
\]

where \( \mu \) is the intercept parameter, \( u_i \) are cluster specific random effects which are assumed to be i.i.d. random variable with density function \( f(u_i) \). Assuming \( t_{ij} \) follows a Weibull distribution,

\[
t_{ij} | \lambda_{ij} \sim \text{Weibull}(\alpha, \lambda_{ij}) \quad (9)
\]
for which the hazard function is,

$$\lambda(t) = \alpha \lambda_{ij} t^{\alpha-1}$$

which is reduced to exponential hazard for $\alpha = 1$

For $j^{th}$ gene in the $i^{th}$ cluster with frailty for $k^{th}$ patient, the proportional hazard frailty model will be,

$$\lambda_{ij}(t_k) = \lambda_0(t) \exp(Z_{ijk}^t \beta + w_{ij})$$

The frailty term $w_{ij}$ is assumed to follow a zero-mean normal distribution with variance $\gamma_{ij}$. The variance term will be used to validate the classification of the gene clusters. Penalized partial likelihood (PPL) method by Breslow is used for the estimation of frailty parameter.

### 2.3 Auto-Regressive Longitudinal Model

In longitudinal study, often the interest lies on the change in observations where the observations are correlated based on time of sampling. In cross-sectional data, the value of a response at a particular time-point may depend on its value at previous time-points. To address this problem, it is required to incorporate an auto-regressive dependency in modeling.

Efforts have been made to include auto-regressive error structure in mixed effect model. The most common formulation based on a continuous-valued latent process assumes that the individual effects follow an Autoregressive model of order 1 (AR(1)) [25], [26]. This model is referred as Latent Autoregressive (LAR) Model. Bacci et al. has described an auto-regressive time-dependent latent process for each subject in the LAR model [27]. They proposed a model for longitudinal data which is based on a mixture of continuous AR(1) processes with different means and correlation coefficients but equal variances. Models based on discrete latent process (Latent Markov (LM) model) assume that the individual effects follow a first-order Markov model. Korn et al. have considered the relation between change in exposure variables to changes in outcome over time with binary outcomes [28]. In their analysis, the number of time points with information is large relative to the number of exposure variables. The same authors have also extended this problem for continuous outcome variables. Rosner et al. used an autoregressive structure over the responses where the observations at a particular time are linearly dependent over observations on earlier time-points [29]. Conditional distribution of $y_{it}$ on $y_{i,t-s}$ for $i^{th}$ subject has been discussed for equi-spaced time points which is later extended to unequispaced time-points [30].
The Autoregressive model of order 1 for longitudinal data is defined as,

$$y_{it} = \alpha + \gamma y_{i,t-1} + \sum_{j=1}^{J} \beta_j x_{ijt} + \sum_{k=1}^{K} \beta_k^* z_{ik} + e_{it}$$  \hspace{1cm} (12)$$

where $i=1,2,\ldots,n$; $t=1,\ldots,T$; $y_{it}$=value of the outcome variable for the $i^{th}$ individual at the $t^{th}$ follow-up, $x_{ijt}$=value of the $j^{th}$ time-dependent covariate for the $i^{th}$ person at time $t$. $z_{ik}$=value of the $k^{th}$ fixed covariate for the $i^{th}$ person, and $e_{it}$ are statistically independent for all $i,t$ with a common distribution $N(0,\sigma^2)$.

### 2.4 Joint Modeling of Longitudinal and Survival Data

#### 2.4.1 Using Auto-regressive-Type model for longitudinal data

In our study, an ‘Auto-regressive type’ model is fitted to the longitudinal data where the final outcome is dependent only on the baseline observations. Proper care is taken to obtain the baseline observations which are considered as error-free. Hence the final observations are compared with the baseline observations to observe the changes and make necessary inference on the current status on the basis of baseline data. So, the model adopted in our study is,

$$y_{i,f} = \alpha + \beta_y y_{i,b} + X_{i,b}\beta_l + Z_{i,b}b_l + e_i = \alpha + \begin{bmatrix} \beta_y \\ \beta_l \end{bmatrix} \begin{bmatrix} y_{i,b} \\ X_{i,b} \end{bmatrix} + Z_{i,b}b_l = M_i + e_i$$  \hspace{1cm} (13)$$

where $y_{i,f}$ is the final observation of the $i^{th}$ patient, $y_{i,b}$ is the baseline observation and $e_i$ is the subject specific error. $X_{i,b}$ and $Z_{i,b}$ are the fixed and random covariates for $i^{th}$ patient at baseline with corresponding coefficients $\beta_l$ and $b_l$. $M_i$ is the true unknown baseline profile.

The proportional hazard submodel for survival data is defined as,

$$\lambda_{ij}(t) = \lambda_0(t) \exp(Z_{ijg}\beta_s + \gamma m_i)$$  \hspace{1cm} (14)$$

where $\gamma$ is the association parameter, $M_i(t) = \{m_i(s),0 \leq s \leq t\}$ is the true unknown longitudinal profile up to time $t$. $Z_{ijg}$ is covariates for survival model and $\beta_s$ is their parameters.

The estimation procedure for joint modeling falls either frequentist approach or Bayesian approach. Both the approaches use the full joint likelihood derived from the joint distribution of repeated measurements and time-to-event data. In this article, our focus was mainly on the Bayesian methodology. The posterior distributions of the model parameters are derived using the standard Markov Chain
Monte Carlo Method (MCMC). Here joint modeling as described by [37] will be followed to perform the necessary analysis. Under this methodology, the sequence of measurements for the $i^{th}$ subject at times $s_{i1}, s_{i2}, ..., s_{in}$ is modeled as

$$y_{ij} = \mu_i(t_j) + W_{1i}(s_{ij}) + \epsilon_{ij}$$  \hspace{1cm} (15)

where $\mu_i(t) = X_{1i}(t)\beta_1$ is the mean response and $W_{1i}(s) = Z_{1i}U_i$ is the subject-specific random effects and $\epsilon_{ij} \sim N(0, \sigma^2_\epsilon)$ is the mutually independent measurement error. The $U_i$ are the vectors of random effects corresponding to the variables $Z_{1i}(s)$. For the survival part, in a weibull model, the survival time for the $i^{th}$ subject follows Weibull($r, \mu_i(t)$) where $\log(\mu_i(t)) = X_{2i}(t)\beta_2 + W_{2i}(t)$ and $r > 0$. The form of $W_2(t)$ is similar to that $W_1(t)$ with subject-specific covariate effect and an intercept term, called as frailty. Therefore the weibull survival model is,

$$\lambda_i(t) = rt^{r-1}exp(X_{2i}(t)\beta_2 + W_{2i}(t))$$  \hspace{1cm} (16)

In joint model, a zero mean bivariate Gaussian model for $(W_{1i}(t), W_{2i}(t))^t$ is assumed which is independent for different subjects. The joint model links the longitudinal and survival models by assuming,

$$W_{1i}(s) = U_{1i} + U_{2i}s$$  \hspace{1cm} (17)

and

$$W_{2i}(t) = \gamma_1 U_{1i} + \gamma_2 U_{2i} + \gamma_3 (U_{1i} + U_{2i}t) + U_{3i}$$  \hspace{1cm} (18)

The parameters $\gamma_1, \gamma_2, \gamma_3$ measures the association between two submodels by associating random intercepts, slopes and fitted repeated measurement at the event time $W_{1i}(t)$ respectively. The prior specifications for the parameters are followed as explaind in [39]. The paired latent variables $(U_{1i}, U_{2i})^t$ has a zero-mean bivariate Gaussian distribution $N(0, \Sigma)$ and $U_{3i}$, independent of $(U_{1i}, U_{2i})^t$ are independent frailty terms modeled as iid $N(0, \sigma^2_3)$. We have obtained the posteriors distributions using Expectation-Maximization algorithm and gaussian prior for the parameters and model selection is performed based on Watanabe-Akaike information criteria (WAIC) [40].

2.4.2 Using Mixed Effect Model for longitudinal data

Joint modeling is an important tool to accomodate both longitudinal and survival observations with time-dependent covariates. Both longitudinal and survival models are associated in a single model with an association parameter to estimate the effect of baseline covariates as well as time-dependent covariates on the disease progression. A classical linear mixed effect model is popularly used to address the
longitudinal study whereas proportional hazard model are widely used for the survival submodel. Research has been extended to a number of different submodels for both longitudinal and survival parts whereas different distributions have been considered for to accommodate various challenges observed in real life data.

In many clinical studies, it is common that both longitudinal measurement data and time-to-event data are collected during follow-up [31]. This has been an active research area for the past two decades. The literature on this topic is extensive, with comprehensive reviews given by [32], [33]. Reviews specific to joint latent class models and multivariate longitudinal data are given by [34] and [35] respectively. In joint models, a classical linear mixed effect model is popularly used to specify longitudinal measurements and Cox proportional hazard model for the survival part. The mixed effect model for longitudinal measurements is associated inside the survival model with an link parameter [36], [37], [38].

For analysing longitudinal measurements, one of the most popular tools is mixed effect model where the response variable is decomposed into two parts, one incorporates the fixed effect covariates whereas the other one explains the random components. The mixed effect model is defined as,

\[ Y_{ij} = M_{ij} + \epsilon_{ij} = X_{ij}\beta + Z_{ij}b + \epsilon_{ij}, \quad \epsilon_{ij} \sim N(0, \sigma^2) \]  \hspace{1cm} (19)

where \( Y_{ij} \) denotes the \( j^{th} \) measure of \( i^{th} \) patient, \( M_{ij} \) is the true effect of the data or, the true longitudinal response which we will model in mixed effect model, \( \epsilon_{ij} \) is the error component attached to the data. \( X_{ij} \) & \( Z_{ij} \) are the fixed effects and random effects respectively. Also, \( \beta \) & \( b \) are the coefficients of fixed effect and random effects respectively. We assume the measurement error component \( \epsilon_{ij} \) follows a Normal distribution with mean 0 and variance \( \sigma^2 \) and the error is independent of \( b \). The estimates of the coefficients can be computed using generalized estimating equations using independent model and also considering unstructured model for the correlation structure and estimate of the correlation components.

The Cox proportional hazard function is described in equation (14). The joint model is defined as,

\[ h_i(t|K_i, M_{ij}) = h_0(t)exp(K_i\alpha + M_{ij}\phi) \]  \hspace{1cm} (20)

where \( K_i \) denotes the time-independent covariates with coefficients \( \alpha \) and \( \phi \) measures the effect of longitudinal process on the survival outcome. The longitudinal submodel is replaced in \( m_{it} \) as before with an association parameter and Bayesian methodology as explained in 2.4.1 is used to obtain posterior distributions of the parameters.
2.4.3 Prognostic Score

In survival analysis, it is important to assign a value or score to a patient based on several factors associated with the survival. This score is generally referred as Prognostic score. Patients with complete information of the factors are included for calculation. Descriptive statistics are evaluated by utilizing Cox proportional hazard model for this group of patients and survival durations are predicted by the utilizing the prognostic factors. The scores from each factor are summed into a raw score for survival prediction. Brier score is one of the popularly used score function which measures the accuracy of probabilistic predictions. The Brier score is a weighted average of the squared distances between the observed survival status and the predicted survival probability of a model. Roughly the weights correspond to the probabilities of not being censored. Prediction error curves are obtained when the Brier score is followed over time. Based on the hazard ratio of proportional hazard model for a particular gene, we compute the contribution of that gene over the survival of patient. For a new patient, those gene expressions are collected and their scaled value are multiplied with the percentage contribution which results in a score value. Those score values are added to get a consolidated score which is a prognostic score. Based on the prognostics score, we compute Brier score to draw inference regarding patient survival.

3 Application on Gene Expression Data

3.1 Description of Data

To perform our procedure, we have used a published dataset on Gene Expression study collected from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE 65622. The data was first published in April, 2016 and later updated in August 2016. All the patients were given neoasjuvant chemotherapy (NACT) followed by chemoradiotherapy (CRT) and treatment evaluation was carried out for four weeks after CRT completion. After 2-4 weeks, radical pelvic surgery was considered. The study consists dataset on 80 patients and the observations were obtained in the following manner. Serum samples were collected at baseline (Sampling point=1), following NACT post-NACT(Sampling point=2), at CRT completion (Sampling point=3), post-CRT (Sampling point=4) and at treatment evaluation (Sampling point=5). With a high-density antibody array (AHH-BLG-1;RayBiotech Inc.), the samples were analyzed for the presence of 507 proteins. Further explanation about the study setting and deatiled description of the data can be cited from Meltzer et al. (2016). The objective of the study is to classify the proteins according to
their effect on cancer progression and investigate the association between factors regarding tumor severity and duration of disease reoccurrence.

3.2 Analysis

In the analysis, we define the event as a relapse of disease or metastatic disease. The patients enter the study at different time points and observed till a common censoring data which is 08/08/2013. The interest of the study is to classify the genes based on their effect on occurrence of local relapse or occurrence of metastatic disease. In case of occurrence of both the diseases, whichever is earlier is considered for event occurrence. Here, 1 indicates the occurrence of disease and 0 indicates non-occurrence. The gene expressions also contain a few missing values which are not considered in the analysis.

The occurrence of event is associated with the gene expression and hence it is required to find the optimal threshold level of activity of gene expression at each time-point. To achieve that, we randomly classify the gene expression at a time point into two parts using Deciles e.g., the expressions under \( p^{th} \) decile is compared the expressions above \( p^{th} \) decile along with the number of events. The survival duration is considered as the duration between two sampling points. If the event occurred in that duration, then the duration is split in two parts. As for example, if an event occurred in between 2\(^{nd}\) and 3\(^{rd}\) sampling data, the duration is considered as 2\(^{nd}\) sampling date to date of occurrence and date of occurrence to 4\(^{th}\) sampling date. For events occurring after the last sampling date, the last duration is considered as last sampling date to date of occurrence.

The comparison is made based on Cox Proportional Hazard. The CoxPH is applied on the two random parts created based on deciles and the p-values of the model is compared for different deciles. The threshold with lowest p-value indicates the optimal threshold value of gene expression for event occurrence. During the formation of parts, it is required to keep an eye on the number of events in the partitions. So, we consider a minimum of 3 event occurrences in each of the partition. Thus for 4 time-points, we obtain 4 threshold values with minimum p-values at each time-point, minimum frailty values and corresponding coefficients. Based on the threshold values and coefficients obtained of the gene expressions, the Progressive, Regressive and Volatile genes are classified. We obtain 86 Progressive genes, 16 Regressive genes and 404 Volatile genes. The correlation network diagram of the genes for different clusters are plotted in figure 3.

We consider both PH and AFT model to obtain the frailty terms for each gene. The patient-specific frailty term is assumed to follow zero-mean Gaussian distribution. The weibull distribution is considered for the response \( y \) i.e. \( \log T_i \). Comparisons are made on the basis of the histograms of variance component of the random
effects for 3 clusteres. It is found that the sd of the frailty components for both tha model are quite similar. The coefficient of variations of the starand deviations for each clusteres are also calculated for comparisons. We ranked the genes based on the variance of the random components obtained in frailty models. The results are shown in table 1 and 2. It is evident that for both the clusters, there are several genes which fall in the top 10 under both AFT and PH frailty. From the histogram plots of standard deviations of frailty components shown in figure 4, we can find a clear distinguish between the patterns of Progressive and Regressive genes. In plotting the histogram for Progressive genes, one gene has frailty variance of 17.625. To get a clear picture of the pattern, that gene is omitted from the plot.

In the next stage, we fit joint models using auto-regressive-type model for longitudinal data and proportional hazard model for time-to-event data for each gene expressions. In medical context, it is important to correlate the final outcome with the baseline observations as the baseline responses are considered to be mostly error-free. The diagnosis of disease and gene classification is better associated with the baseline observations. We consider all those observations for which the baseline observations are present and there are atleast 2 longitudinal observations are found. For modeling of longitudinal observations of each gene, we considered only the baseline observation and final outcome for a patient. The survival duration is considered as the duration till occurrence of event or censoring. The covariates considered were T-stage (0 - tumor not found, 1-4 - Size of the tumor, higher the number, greater the size), N-stage (the number of nearby lymph nodes that contains cancer. Higher the value, larger the spread) and TRG-score (Tumor Regression Grading Score. 0 - Complete Regression, 5- Absence of Regression). Thus we fit a Bayesian joint model with auto-regressive type longitudinal model with gaussian response and weibull survival duration with r=1 using R-INLA. Among different fitted models, the best model chosen on the basis of WAIC is,

\[
y_{ij} = \beta_{11} + \beta_{12}s_{ij} + \beta_{13} \times (t_{stage})_i + W_{1i}(s_{ij}) + \epsilon_{ij}
\]  

(21)

where \( W_{1i}(s_{ij}) = U_{11i} + U_{12i}s_{ij} \) and 

\[
\log(\mu_i) = \beta_{21} + \beta_{22} \times (t_{stage})_i + W_{2i}(t)
\]  

(22)

where \( W_{2i}(t) = \gamma_1 U_{11i} + \gamma_2 U_{21i} + \gamma_3 (U_{12i} + U_{22i}t) + U_{3i} \). The prior distribution of \( U_{11i} \) and \( U_{21i} \) is specified as \( N \left( 0, \begin{pmatrix} 3 & 10 \\ 10 & 0 \end{pmatrix} \right) \) and the prior distribution of \( U_{11i} \) and \( U_{21i} \) is specified as \( N(0, 0.01) \).

The same is performed for mixed effect longitudinal model also using t-stage and n-stage for longitudinal covariates and t-stage for survival covariate. Among different fitted models, the best model chosen on the basis of WAIC is,

\[
y_{ij} = \beta_{11} + \beta_{12}s_{ij} + \beta_{13} \times (t_{stage})_i + W_{1i}(s_{ij}) + \epsilon_{ij}
\]  

(23)

14
where \( W_{1i}(s_{ij}) = U_{11i} + U_{12i}s_{ij} \) and

\[
\log(\mu_i) = \beta_{21} + \beta_{22} \times (t_{stage})_i + W_{2i}(t)
\]  \hspace{1cm} (24)

where \( W_{2i}(t) = \gamma_1 U_{11i} + \gamma_2 U_{21i} + \gamma_3 U_{12i} + U_{3i} \). The prior specifications are followed same as previous. The results for both joint models is shown in table 3.

We conside 10 genes from each of the Progressive genes and regressive genes based on the minimum frailty for calculation of prognostics scores. The contributions of genes on the survival is also computed using hazard ratio of Cox proportional hazard model shown in table 4. 12 patients with complete information was obtained and 5 of them have experienced the event. The prognostic scores of those patients are calculated as shown in the table and based on that, we obtain the Brier plot. 4 different Cox models are compared to obtain the best prediction model. The covariates considered for the models are (i) Both progscore 1 and progscore 2 (Reference) (ii) only progscore 1 (Cox Model 1), (iii) only progscore 2 (Cox Model 2), (iv) the sum value of progscore 1 and progscore 2 (Cox Model 3). The progscore 1 denotes score based on only the Progressive Genes and progscore 2 denotes score based on only the Regressive Genes. It is evident from the figure that the Cox model based on only the Progressive genes have the lowest prediction error among them.

4 Discussion and Conclusion

Identification of biomarkers is an important aspect in oncology research. Early detection of cancer is crucial to initiate treatment at early stages and improve overall survival rate. Currently the tissue-based biopsy, which has a potential detrimental effect on patients, is typically used for detection of tumor. So, plasma/serum biomarkers are becoming more popular due to their potential in cancer screening and minimal-invasive nature. There are several available methods to detect biomarkers which have the potential to diagnose disease progression and for accurate prognosis. In most of the times, it is found that those analytically sound biomarkers fails to pass proper validation tests in medical process. As a result, though there are a number of procedures available for biomarker detection, a very few number of markers are really promoted to achieve the ‘status’ of biomarker. The problem becomes more challenging for high-dimensional data.

In this article, we described an efficient statistical procedure of filtration method and ranking of the protein markers based on their effect in disease progression from high-dimensional data. We identified a few protein biomarkers and further explained the use of different types of joint modeling using those biomarkers to present the applicability of our work. It is pivotal to identify the nature of influence
of protein markers on overall survival. However, due to the data-driven approach, there is always a chance of plausible randomness in the modeling. In this study, we developed a proper statistical algorithm which produces clustering of genes based on their effect on disease progression and also ranking of genes based on the presence of random component when information of a large number of markers are available. Also, we assigned prognostic scores to few selected patients based on the contribution of identified biomarkers on survival outcome.

There are good scopes for further improvements that can be carried out on the proposed procedure. The proposed ‘auto-regressive type’ joint modeling can be refined more efficiently. Several distributional assumptions can be considered for responses and parameters to accommodate different medical situations. Also the methodology can be extended for binary marker responses. There is an excellent scope of refinement in the filtration method using different survival models and accommodating different types of censoring methods. The procedure can be further extended for competing risks to consider multiple events of interest in context of survival. Also, use of bayesian methodology in the clustering algorithm can be an interesting area of study.

In conclusion, the next generation of liquid biopsy studies is crucial for establishing clinical applicability of blood-based cancer diagnostics and improvement of survival rates. We are optimistic that the proposed methodology for discovery of new biomarkers will decrease the cost of treatment and will show the light for better methods to detect disease at more treatable stages.

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6 Supplementary Material

The detailed R programming code used in this article is available in the following link [https://github.com/souvikbanerjee91/gene_expression.git](https://github.com/souvikbanerjee91/gene_expression.git)

7 Declaration of Interest

None
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Figure 2: Brier plot for different prognostic models
(a) Correlation Network on Progressive Genes 1-20 alphabetically

(b) Correlation Network on Regressive Genes

Figure 3: Correlation Network of Progressive and Regressive Genes. Red for negative correlation and green for positive correlation. The thickness defines strength of correlation.
Figure 4: Histogram of standard deviation of frailty component obtained using two different models. Cluster 1, 2 and 3 represents Progressive, Regressive and Volatile Genes respectively.
Table 1: Coefficients of fitted AFT model and Standard Deviation of random components of top 10 Markers according to ascending order of randomness (a) For Progressive genes (cluster=1), (b) For Regressive Genes (cluster=2)

| Gene Name          | Gene No. | intercept | coefficient of expression value | sd of AFT frailty | range    | sd/range | cluster |
|--------------------|----------|-----------|---------------------------------|-------------------|----------|----------|---------|
| TGF-beta RIII      | 455      | 9.001     | -0.024                          | 0.831             | 79.722   | 0.010    | 1       |
| DAN                | 93       | 9.188     | -0.012                          | 1.010             | 343.947  | 0.003    | 1       |
| APJ                | 20       | 9.597     | -0.011                          | 1.148             | 242.469  | 0.005    | 1       |
| S100 A8/A9         | 418      | 9.346     | -0.001                          | 1.191             | 2974.719 | 0.0004   | 1       |
| Lymphotactin / XCL1| 330      | 9.340     | -0.007                          | 1.245             | 385.803  | 0.003    | 1       |
| GDF3               | 171      | 9.219     | -0.001                          | 1.314             | 13525.530| 9.72e-05 | 1       |
| NRG3               | 383      | 8.714     | -0.000                          | 1.315             | 26532.430| 4.96e-05 | 1       |
| GREMLIN            | 192      | 8.473     | -0.000                          | 1.359             | 17648.610| 7.70e-05 | 1       |
| TFPI               | 446      | 8.303     | -0.002                          | 1.388             | 213.524  | 0.006    | 1       |
| Ubiquitin+1        | 492      | 8.150     | -0.000                          | 1.391             | 22814.850| 6.09e-05 | 1       |
| Eotaxin / CCL11    | 117      | 7.857     | 0.001                           | 1.440             | 2127.944 | 0.001    | 2       |
| Activin RIB / ALK-4| 6        | 7.878     | 0.001                           | 1.451             | 2417.925 | 0.001    | 2       |
| Frizzled-4         | 158      | 7.746     | 0.002                           | 1.512             | 762.211  | 0.002    | 2       |
| IL-3               | 291      | 7.571     | 0.001                           | 1.518             | 1587.440 | 0.001    | 2       |
| IL-2 R gamma       | 275      | 7.650     | 0.002                           | 1.524             | 1122.739 | 0.001    | 2       |
| IL-22 BP           | 282      | 7.690     | 0.001                           | 1.528             | 1756.997 | 0.001    | 2       |
| IL-31              | 293      | 7.846     | 0.001                           | 1.581             | 3417.479 | 0.001    | 2       |
| IL-2 R beta /CD122 | 274      | 7.865     | 0.001                           | 1.596             | 680.924  | 0.002    | 2       |
| Insulin            | 307      | 8.266     | -9.32e-05                       | 1.632             | 3613.603 | 0.001    | 2       |
| GDF1               | 169      | 8.790     | -0.001                          | 1.864             | 734.911  | 0.003    | 2       |
Table 2: Coefficients of fitted PH model and Standard Deviation of random components of top 10 Markers according to ascending order of randomness (a) For Progressive genes (cluster=1), (b) For Regressive Genes (cluster=2)

| Gene Name               | Gene No. | coefficient of expression value | sd of PH frailty | range   | sd/range | cluster |
|-------------------------|----------|----------------------------------|------------------|---------|----------|---------|
| TGF-beta RIII           | 455      | 0.024                            | 0.410            | 79.723  | 0.010    | 1       |
| MMP-19                  | 360      | 0.005                            | 0.619            | 4269.286| 0.000    | 1       |
| Dkk-3                   | 98       | 0.006                            | 0.634            | 441.068 | 0.003    | 1       |
| CLC                     | 71       | 0.003                            | 0.635            | 507.079 | 0.003    | 1       |
| S100 A8/A9              | 418      | 0.001                            | 0.677            | 2974.719| 0.001    | 1       |
| GREMLIN                 | 192      | 0.000                            | 0.692            | 17648.610| 7.70e-05| 1       |
| DAN                     | 93       | 0.011                            | 0.697            | 343.947 | 0.003    | 1       |
| TLR4                    | 472      | 0.009                            | 0.764            | 522.589 | 0.003    | 1       |
| Lymphotactin / XCL1     | 330      | 0.007                            | 0.779            | 385.803 | 0.003    | 1       |
| APJ                     | 20       | 0.010                            | 0.802            | 242.470 | 0.005    | 1       |

| Gene Name               | Gene No. | coefficient of expression value | sd of PH frailty | range   | sd/range | cluster |
|-------------------------|----------|----------------------------------|------------------|---------|----------|---------|
| Activin RIB / ALK-4     | 6        | -0.001                           | 0.900            | 2417.925| 0.001    | 2       |
| Eotaxin / CCL11         | 117      | -0.001                           | 0.943            | 2127.944| 0.001    | 2       |
| IL-2 R gamma            | 275      | -0.001                           | 1.071            | 1122.739| 0.001    | 2       |
| IL-22 BP                | 282      | -0.001                           | 1.074            | 1756.997| 0.001    | 2       |
| IL-3                    | 291      | -0.001                           | 1.096            | 1587.440| 0.001    | 2       |
| Insulin                 | 307      | -0.001                           | 1.133            | 3613.603| 0.001    | 2       |
| IL-31                   | 293      | -0.001                           | 1.134            | 3417.479| 0.000    | 2       |
| Frizzled-4              | 158      | -0.002                           | 1.172            | 762.211 | 0.002    | 2       |
| IL-2 R beta /CD122      | 274      | -0.001                           | 1.200            | 680.924 | 0.002    | 2       |
| FGF-12                  | 135      | -0.001                           | 1.201            | 2917.189| 0.001    | 2       |
Table 3: Bayesian Joint Model output for longitudinal and survival model. The first table shows for auto-regressive-type longitudinal model and the second table shows for mixed effect longitudinal model. For both the cases, exponential survival model has been used.

|                                | mean  | sd    | 0.025 Quantile | 0.5 Quantile | 0.975 Quantile |
|--------------------------------|-------|-------|----------------|--------------|---------------|
| Intercept ($\beta_{11}$)       | 22.057| 6.629 | 9.041          | 22.056       | 35.061        |
| Intercept ($\beta_{21}$)       | -12.831| 5.118 | -22.686        | -12.818      | -2.954        |
| baseline covariate ($\beta_{12}$) | 0.723 | 0.078 | 0.570          | 0.722        | 0.875         |
| Precision for the Gaussian observations | 10562.321 | 58.357 | 10448.069 | 10562.153 | 10677.637 |
| Precision for U11 (component 1) | 0.001 | 0.000 | 0.001          | 0.001        | 0.001         |
| Precision for U11 (component 2) | 0.006 | 0.000 | 0.006          | 0.006        | 0.006         |
| Covariance ($\rho_{12}$) for U11 | -0.110 | 0.002 | -0.113        | -0.109       | -0.106        |
| Precision for U3 ($\sigma^2$)  | 65.726 | 33.222 | 6493.662 | 6558.638 | 6624.353 |
| Precision for U12              | -0.111 | 0.001 | -0.013        | -0.011       | -0.009        |
| Precision for U22              | -1.958 | 0.003 | -1.964        | -1.958       | -1.951        |

|                                | mean  | sd    | 0.025 Quantile | 0.5 Quantile | 0.975 Quantile |
|--------------------------------|-------|-------|----------------|--------------|---------------|
| Intercept ($\beta_{11}$)       | 0.048 | 0.385 | -0.708        | 0.048        | 0.8046        |
| Intercept ($\beta_{21}$)       | -1.986| 0.573 | -3.114        | -1.985       | -0.8636       |
| time ($\beta_{12}$)            | 0.012 | 0.137 | -0.257        | 0.012        | 0.2815        |
| t_stage ($\beta_{13}$)         | 0.030 | 0.119 | -0.204        | 0.030        | 0.2638        |
| n2tage ($\beta_{14}$)          | 0.040 | 0.226 | -0.403        | 0.04         | 0.483         |
| t_stage ($\beta_{22}$)         | -0.125| 0.172 | -0.463        | -0.125       | 0.2109        |
| Precision for the Gaussian observations | 0.000 | 0.000 | 0.000          | 0.000        | 0.000         |
| Precision for U11 (component 1) | 0.001 | 0.000 | 0.001          | 0.001        | 0.001         |
| Precision for U11 (component 2) | 0.003 | 0.000 | 0.003          | 0.003        | 0.003         |
| Covariance ($\rho_{12}$) for U11 | 0.944 | 0.001 | 0.942         | 0.946        | 0.946         |
| Precision for U3 ($\sigma^2$)  | 132.752 | 2.255 | 128.345 | 132.744 | 137.214 |
| Precision for U12              | -1.242 | 0.015 | -1.273        | -1.242       | -1.213        |
Table 4: Percentage Contribution on Survival of patients by prognostic genes using Progressive genes (cluster=1) and Regressive Genes (cluster=2)

| Gene Name                      | Threshold Value | Regression Coefficient | Range Value | Minimum Value | Cluster | Hazard Ratio | Percentage Contribution |
|--------------------------------|-----------------|------------------------|-------------|---------------|---------|--------------|-------------------------|
| APJ                            | 87.917          | 0.524                  | 242.470     | 35.445        | 1       | 1.005        | 0.099                   |
| DAN                            | 46.968          | 0.698                  | 129.225     | 27.347        | 1       | 1.014        | 0.100                   |
| GDF3                           | 3260.853        | 0.299                  | 13525.530   | 726.510       | 1       | 1.000        | 0.099                   |
| GREMLIN                        | 1152.626        | 1.060                  | 17648.610   | 395.198       | 1       | 1.000        | 0.099                   |
| Lymphotactin / XCL1            | 132.482         | 0.333                  | 264.899     | 70.644        | 1       | 1.007        | 0.099                   |
| NRG3                           | 1226.503        | 1.022                  | 26396.300   | 508.641       | 1       | 1.001        | 0.099                   |
| S100 A8/A9                     | 613.560         | 0.606                  | 2315.253    | 369.818       | 1       | 1.000        | 0.099                   |
| TFPI                           | 200.761         | -0.593                 | 164.217     | 19.129        | 2       | 0.997        | 0.100                   |
| TGF-beta RIII                  | 56.306          | 1.211                  | 60.818      | 13.441        | 1       | 1.000        | 0.099                   |
| Ubiquitin+1                    | 346.890         | 0.694                  | 7658.155    | 220.388       | 1       | 1.000        | 0.099                   |
| Activin RIB / ALK-4            | 393.051         | -0.566                 | 1825.192    | 214.306       | 2       | 0.999        | 0.100                   |
| Eotaxin / CCL11                | 162.370         | -0.495                 | 2127.944    | 95.435        | 2       | 0.999        | 0.100                   |
| Frizzled-4                     | 182.803         | -0.297                 | 762.211     | 72.907        | 2       | 0.997        | 0.100                   |
| GDF1                           | 476.263         | -0.470                 | 674.048     | 127.159       | 2       | 0.999        | 0.099                   |
| IL-2 R beta /CD122             | 219.408         | -0.631                 | 538.898     | 162.753       | 2       | 0.998        | 0.100                   |
| IL-2 R gamma                   | 200.761         | -0.593                 | 1095.012    | 115.514       | 2       | 0.999        | 0.100                   |
| IL-22 BP                       | 633.122         | -0.407                 | 1678.951    | 168.434       | 2       | 0.999        | 0.100                   |
| IL-3                           | 341.101         | -0.717                 | 1394.570    | 226.991       | 2       | 0.999        | 0.100                   |
| IL-31                           | 293.513         | -0.348                 | 3093.107    | 61.015        | 2       | 0.998        | 0.100                   |
| Insulin                        | 1452.828        | -0.526                 | 2959.707    | 191.915       | 2       | 0.999        | 0.099                   |