Evaluation of Time-Varying Biomarkers in Mortality Outcome in COVID-19: an Application of Extended Cox Regression Model

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ABSTRACT

Background: COVID-19 pandemic has created many challenges for clinicians. The monitoring trend for laboratory biomarkers is helpful to provide additional information to determine the role of those in the severity status and death outcome. Objective: This article aimed to evaluate the time-varying biomarkers by LOWESS Plot, check the proportional hazard assumption, and use to extended Cox model if it is violated. Methods: In the retrospective study, we evaluated a total of 1641 samples of confirmed patients with COVID-19 from October until March 2021 and referred them to the central hospital of Ayatollah Rohani Hospital affiliated with Babol University of medical sciences, Iran. We measured four biomarkers AST, LDH, NLR, and lymphocyte in over the hospitalization to find out the influence of those on the rate of death of COVID-19 patients. Results: The standard Cox model suggested that all biomarkers were prognostic factors of death (AST: HR=2.89, P<0.001, Lymphocyte: HR=2.60, P=0.004, LDH: HR=2.60, P=0.006, NLR: HR=1.80, P<0.001). The additional evaluation showed that the PH assumption was not met for the NLR biomarker. NLR biomarkers had a significant time-varying effect, and its effect increase over time (HR(t)=exp (0.234+0.261×log(t)), p=0.001). While the main effect of NLR did not show any significant effect on death outcome (HR=1.26, P=0.097). Conclusion: The reversal of results between the Cox PH model and the extended Cox model provides insight into the value of considering time-varying covariates in the analysis, which can lead to misleading results otherwise. Keywords: LOWESS plot, Cox PH model, Proportional hazard assumption, extended Cox model, COVID-19 dataset.

1. BACKGROUND

The ongoing coronaviruses (COVID-19) epidemic, first reported from Wuhan, China in December 2019, has so far caused more than 505 million confirmed cases and more than 6.2 million deaths worldwide (1). Some of the factors that are suspected to be responsible for the severe respiratory failure and death are affected by demographics (e.g., age, sex, comorbidities (e.g., kidney disease, cardiovascular disease, liver injury, hypertension, obesity...), and clinical measures (e.g., body mass index (BMI) (2-4)). Also, many studies suggest that the biomarkers are one of the main factors underlying disease severity and death (5, 6). Review studies have investigated the effect of laboratory markers and blood routine parameters in predicting the progression of this infectious disease (7-9). Survival studies have been widely used in COVID-19 to evaluate the effect of biomarkers on time to severity or ICU admission or mortality, but most of these studies have been related to the baseline value of biomarkers and the use of time-fixed Cox regression models (10, 11). The Cox regression model assumes that the biomarkers reported at one single time point for each patient are sufficient to predict survival. In COVID-19, biomarkers change over time and the course changes of biomarkers can provide more reliable information than baseline values, and estimates of prognosis will be efficient if such time-dependent changes of biomarkers are taken into account in the model (12, 13). Therefore, understanding the changes in these biomarkers over time...
and the trajectory of the disease have a significant role in providing insight into the severity and death of COVID-19. So, to further analyze blood routine biomarkers in COVID-19 disease infection, extended Cox model approaches under the time-varying coefficient have been proposed as an alternative to the Cox proportional hazards model for survival data analysis in long-term follow-up.

2. OBJECTIVE
The main purpose of this study was to evaluate the performance of the extended Cox model when the proportional hazards assumption in Cox model is not satisfied according to biomarkers changes during hospitalization.

3. PATIENTS AND METHODS
Subjects and Data collection
A retrospective study of historical cohort was conducted that included a sample of 1667 patients of COVID-19 referring to the Ayatollah Rohani Hospital that is affiliated to Babol University of Medical Sciences from 22 October 2020 to 5 March 2021. This hospital is the main referral center for COVID-19 in Babol the North of Iran. The data were obtained from the electronic hospital information system (HIS). Demographic and clinical characteristics, including age, gender, severity status, and comorbidity (cardiovascular, kidney disease, and liver injury) and trajectory biomarkers (laboratory examination) included an AST, LDH, NLR, and Lymphocyte count were extracted. Data were analyzed as survival method; the length of hospitalization was considered as a time variable (T). Patients’ death was also regarded as an event, Recovery, discharge with personal consent, and transferring to other hospitals were considered as censorship. We excluded the patients under the age of 18 years and patients who markers follow-up was measured in less than one day. As shown in Figure 1, 1641 cases of COVID-19 were entered into the analysis. This study was approved by Babol University of medical science ethics committee (Ethic code: IR.MUBABOL.REC.1400.204).

Statistical analyses
In data analysis, compare the patients’ characteristics, categorical data were presented as frequency and analyzed using chi-square test. Continuous variables were summarized by the median, interquartile range (IQR) and analyzed using Mann-Whitney rank test when the data were non-normally distributed. Otherwise, the independent t-test was used. Dynamic changes of biomarkers levels by severity status were depicted using Locally Weighted Scatterplot Smoothing (LOWESS). The Cox proportional hazard regression models adjusted by age, sex, severity status, and comorbidity disease (cardiovascular, kidney disease, and liver injury) were used to determine the relationship between biomarkers levels and the event of death. Graphical method and scaled Schoenfeld residuals used to confirm the model support the assumption of proportionality. For those biomarkers that hazard ratio was not constant over time and the proportional hazard assumption was not fulfilled, the extended Cox model was used for analysis. Finally, we used Akaike information’s criterion (AIC) for the selection of the best model to compare the standard Cox regression model and extended Cox regression model.

Cox proportional hazard model
The equation of Cox regression model is written as follows:

\[ h(t \mid X) = \lambda_0(t) \exp \left( \sum_{i=1}^{k} \beta_i X_i \right) \]

And where \( X = (X_1, X_2, ..., X_P) \) is a set of predictive variables. The hazard for individual \( i \) at time \( t \) is the product of the baseline hazard function and a linear function of covariates. In this model, hazard ratio is constant at all times, in other words, it does not depend on time (1).

Extended Cox model
If hazard ratio would be not constant over time and the hazard proportionality assumption does not satisfy, they may lose power. Therefore, the extended Cox model must be used (1).

An extended Cox proportional hazards model consider as follows:

\[ h(t \mid X, Z) = \lambda_0(t) \exp \left( \sum_{i=1}^{k+1} \beta_i X_i + \sum_{j=1}^{p} \theta_j X_j(t) \right) \]

Where \( Xi \) is a covariate that has time independency and \( Xf \) is a covariate that has time dependency.

The time-varying covariate is \( X_j(t) = X_j \cdot g_j(t) \), where \( g_j(t) \) is defined as a time function for covariate-\( j \). The time function can be considered as forms \( X_j(t) = t \) and \( X_j(t) = \ln(t) \).

Assessment of Proportional Hazards Assumption
There are popular approaches for confirming that a model supports the assumption of proportionality (graphical method, scaled Schoenfeld residuals, and adding time-dependent covariate). In this article, we used graphical method and scaled Schoenfeld residuals.

The test related to the scaled Schoenfeld residuals used to assess correlate between residuals and time. The proportional hazard assumption is met if there is a non-significant relationship between residuals and time, in other words, the straight horizontal line has a zero slope (15). A graphical method using log (-log (survival)) curves corresponding to two different groups defined as

\[ n \left[ -\ln S(t, x_1) \right] - n \left[ -\ln S(t, x_2) \right] = \beta (x_1 - x_2), \]

log (-log (survival)) plot versus survival time for two groups would see parallel if the proportional hazard is constant (1, 2).

Selection of the Best Models
The AIC is given by AIC = -2 log (likelihood) +2P and BIC is given by BIC = -2 log (likelihood) +p log (n), where \( p \) is the number of parameters. The AIC with minimum value has better likelihood and goodness of fit(3).

The software programs R version 3.6.1 and STATA 15 were used for the analyses. A two-tailed p<0.05 show a significant difference.

4. RESULTS
Patients’ Demographic, Clinical, and Laboratory Characteristics
Baseline characteristics and laboratory parameters on admission are shown in Table 1.

A total of 1641 adult patients with COVID-19 were included in the analysis. The median age was 58(IQR, 47-70 years), and 843(51.4%) were male. Among these patients, 900(54.8%)
were non-severe cases and 741 (45.2%) were classified as severe cases. There were differences in the baseline laboratory parameters between severe and non-severe groups. Severe group had higher AST (41 vs 38 U/L; P < 0.001), higher LDH (691 vs 609.2 U/L; P < 0.001), and lower lymphocyte count (18 vs 21 ×10^9/L; P < 0.001). There were no significant differences in gender ratio and NLR between severe group and non-severe group.

Dynamic Profile of Laboratory Findings in Patients with COVID-19

Figure 2 shows a remarkable elevation of LDH level upon admission between two groups, which was rapidly increased and was maintained at higher levels in the severe group at the first 10 days. The level of lymph in both groups was relatively stable at onset and then decreased in the severe group after 15 days.

As shown in the results, the level of AST in both groups was relatively stable at the first 20 days and above the normal range line. Then its level in the severe group was higher than the non-severe group and only in the non-severe group inclined in the normal range after 20 days. Also, NLR level in both groups was relatively stable during the follow-up period and above the normal range line in both groups.

Determining Associations between Biomarkers and Mortality

Hazard ratios (HRs) for the associations between lymphocyte, AST, LDH, and NLR and mortality outcomes are provided using Cox PH model in Table 2. Age, gender, severity (severe group and non-severe group), and comorbidities (cardiovascular, kidney, and liver injury) were adjusted as founders.

In AST, LDH, and NLR biomarkers, elevated values in lymphocyte biomarkers, decreased values were associated with the highest risk of death. In other words, the risk of mortality was significantly increased in patients with abnormal range compared to patients in the normal range.

Plot log-minus-log (survival) for Proportional Hazard Assumption
Figure 3 show that the plot of -log (-log (survival)) for LDH, Lymphocyte, and NLR biomarkers with normal value and abnormal value that curve was parallel, so these biomarkers satisfied the proportional hazard assumption. This figure shows that plot of –log (-log (survival)) AST intersected, then, the –log (-log (survival)) curve for AST is rarely perfectly parallel, it can be said that there is little reason to doubt the fulfillment of the proportional hazard assumption. Furthermore, for more consideration, it can be evaluated by Schoenfeld Residuals.

Evaluated by Scaled Schoenfeld Residuals for Proportional Hazard Assumption

For each biomarker, Schoenfeld residuals were plotted during the time in Figure 4, and tests for zero slope, and the corresponding p-values are reported in Table 3.

Based on Figure 4, the plot of scaled Schoenfeld residuals for AST and NLR biomarkers did not approach the horizontal line or have a slope that did not approach to zero. Thus, the slope different from zero would be evidence against proportionality.

Following the plot based on Schoenfeld residual, we observe LDH and Lymphocyte biomarkers approaches horizontal and have approach zero slopes, therefore, proportional hazard assumption is fulfilled.

Biomarkers that were deemed likely to meet the proportional Hazard assumption with p>0.05 were the LDH, AST, and Lymphocyte, these findings suggest a constant hazard ratio for these biomarkers. Meanwhile, and NLR biomarker was much smaller than 0.05, so it does not meet the proportional hazard assumption.

Based on plot log-minus-log and Schoenfeld residuals it can be inferred that only NLR did not satisfy the assumption of proportional hazard so this study continued to extended Cox model.

Fitting the best model

Because of the violation of the PH assumption for NLR biomarker, extended Cox model with time-dependent variables is needed to overcome the rejection of this assumption. Table 4 shows that the AIC and BIC values of the extended Cox model with time-dependent variable (NLR) are lower than the Cox PH model.

The extended Cox model by including the combination of the time functions g (t) =ln (t) produced an AIC value of 9901.368 while the modeling by including the combination of the time functions g (t) =t produced an AIC value of 9903.479. Thus, the best model for NLR biomarker is extended Cox model with time function g (t) =ln (t) because the model has the smallest score of Akaike information's criterion (AIC), this final model is derived from the results (Table 5).

Prediction of the time to mortality outcome of the patients by using the extended Cox model

According to this model shown in Table 5, the coefficient for NLR×ln (t) is statistically significant, implying that the effect of NLR varies with time. The time-varying effect of NLR can be written as β (t) = 0.234+0.261×log (t). The value of the hazard ratio of NLR was not constant and depended on time. The hazard ratio of death is equal to exp (β (t)) in abnormal NLR as compared with normal group.

Also, severity variable was the significant factor that affected the death outcome in COVID-19 patients (HR=1.933, P<0.001). It means that patient with severe status has a higher risk of death rate of 1.933 times than non-severe group. This is especially true in kidney comorbidity disease (HR=1.858, P=0.004) and age variable (HR=1.031, P<0.001).

5. DISCUSSION

COVID-19 infection, which targets the lungs as the main organ, can cause severe lung injury and may lead to death. Several Studies on COVID-19 have generally been limited to the description of the comorbidities, symptoms, clinical characteristics, and microbiological findings (4-7). To further analyze the blood routine parameters, for the hospitalized COVID-19 cohort, we collected the longitudinal daily

<table>
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<th>Categories</th>
<th>β</th>
<th>HR (95%CI)</th>
<th>SE</th>
<th>p-value</th>
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<tr>
<td></td>
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<tr>
<td>Severity</td>
<td>Non-severe</td>
<td>0.659</td>
<td>1.93(1.64-2.27)</td>
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<td>&lt;0.001</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Comorbidity</td>
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<td>-</td>
<td>-</td>
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<tr>
<td></td>
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<td>0.150</td>
<td>1.16(0.96-1.39)</td>
<td>0.108</td>
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<td></td>
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<td></td>
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<td>-</td>
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<tr>
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<td>normal*ln(t)</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
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<td>0.261</td>
<td>1.29(1.11-1.52)</td>
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Table 5. Estimation of model parameters of extended Cox model with g (t) =ln (t)
Measurement for biomarkers such as NLR, AST, LDH and Lymphocyte. This study recorded information of 1641 discharged patients with COVID-19 which included 741 severe and 900 non-severe. The daily measure of biomarkers levels was represented by LOWESS plot to display the difference in biomarkers levels between severity statuses. LOWESS plot shows, as others have shown, that the Lymphocyte is decreased (8-10) and LDH is increased (11) in individuals with severe COVID-19.

In this article, first, we evaluated the effect of risk factors such as age and sex, severity status and biomarkers levels in baseline on the death outcome using Cox proportional hazard model, similar to some other studies using Cox model. Advanced age, comorbidities, sex (female) and severe group were the risk factors for mortality outcomes (5, 12). Also, a significant relationship was found between all potential biomarkers (including, Lymphocyte, NLR, AST, and LDH) with mortality that is consistent with previous studies (13, 14).

In the Cox proportional model, the hazard ratio is not time-dependent so the ratio of the hazard function to two different individuals’ remains constant at all times. Subsequently, since the time-varying biomarkers were collected in COVID-19 dataset, hazard ratio maybe not constant over time and the PH assumption is violated. Thus, the evaluation of PH assumption by using plot log-minus-log of survival, Plot scaled Schoenfeld residual, and goodness of fit test based on Schoenfeld residuals show that all biomarkers was fulfilled the proportionality assumption except NLR.

In the current study, the extended Cox model was applied by adding time-by-covariate interactions for NLR biomarker which not met the PH assumption in the Cox PH model. Functions of time \( g(t) \) used in interaction term was \( g(t)=t \) and \( g(t)=\ln(t) \) which AIC and BIC value of \( \ln(t) \) function was lower than the \( t \) function. According to the function of \( \ln(t) \), the interaction parameter in extended Cox model was positive, suggesting that the hazard ratio was increasing over time and the estimated hazard ratio for the NLR biomarker was given by: \( HR(t)=\exp(0.234+0.261\times\ln(t)) \). Thus, the hazard ratio was 1.51, 1.72, and 1.82 at respectively 5, 15 and 25 days. NLR biomarker had a significant effect on time-by-covariate interaction and its main effect did not show any significant effect in extended Cox model, while the Cox PH model shows significant effect for NLR. This finding shows that departure from PH assumption can lead to biased in resulting estimates. Thus, extended Cox model is an appropriate model to show the correct effects in these conditions. Also, as shown based on the AIC and BIC criteria, the values of these criteria were lower for the extended Cox model (AIC=9901.368, BIC=9955.424) than for the Cox model (AIC=9909.865, BIC=9957.164) and the extended Cox model can increase the goodness-of-fit of the Cox PH model in mortality rate in COVID-19 data. Therefore, extended Cox regression model as an appropriate model by taking time-varying covariates into account in survival modeling is required to improve the estimate.

A suggestion for future research is to use the stratified Cox regression model.
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Figure 3. Plot log-minus-log survival of AST, LDH, Lymphocyte and NLR biomarkers

Figure 4. Plot of scaled Schoenfeld residuals for different biomarkers
regression. This model is an appropriate extension of the standard Cox models to include the covariates with non-proportional hazards and control for their effects in the model. Also, performing a test based on the cumulative residuals and types of residuals including the martingale, deviance, and score can help to detect PH violation. Identifying other biomarkers affecting mortality with an appropriate model could be the subject of further research. Although our analysis adjusts for the age, sex, comorbidity, and severity status, we have not incorporated therapies’ effect in these patients. In addition, there may be other confounding factors outside the scope of our study.

6. CONCLUSION

These findings suggest that physicians and public health officials should emphasize the importance of measuring biomarkers during hospitalization in COVID-19 pandemic. To control bias in estimates and prevent misleading results, extended Cox model offers the ability to measure markers longitudinally to evaluate results. Our findings show that NLR is more appropriate to be analyzed by extended Cox model rather than Cox proportional hazard model. The clinicians should be taken into consideration any other time-dependent biomarkers in survival analysis.

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