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Case Study

A predictive paradigm for COVID-19 prognosis based on the longitudinal measure of biomarkers

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Abstract

Novel coronavirus disease 2019 (COVID-19) is an emerging, rapidly evolving crisis, and the ability to predict prognosis for individual COVID-19 patient is important for guiding treatment. Laboratory examinations were repeatedly measured during hospitalization for COVID-19 patients, which provide the possibility for the individualized early prediction of prognosis. However, previous studies mainly focused on risk prediction based on laboratory measurements at one time point, ignoring disease progression and changes of biomarkers over time. By using historical regression trees (HTREEs), a novel machine learning method, and joint modeling technique, we modeled the longitudinal trajectories of laboratory biomarkers and made dynamically predictions on individual prognosis for 1997 COVID-19 patients. In the discovery phase, based on 358 COVID-19 patients admitted between 10 January and 18 February 2020 from Tongji Hospital, HTREE model identified a set of important variables including 14 prognostic biomarkers. With the trajectories of those biomarkers through 5-day, 10-day

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and 15-day, the joint model had a good performance in discriminating the survived and deceased COVID-19 patients (mean AUCs of 88.81, 84.81 and 85.62% for the discovery set). The predictive model was successfully validated in two independent datasets (mean AUCs of 87.61, 87.55 and 87.03% for validation the first dataset including 112 patients, 94.97, 95.78 and 94.63% for the second validation dataset including 1527 patients, respectively). In conclusion, our study identified important biomarkers associated with the prognosis of COVID-19 patients, characterized the time-to-event process and obtained dynamic predictions at the individual level.

Key words: COVID-19; longitudinal data; dynamic risk prediction; time-to-event

Introduction

The coronavirus disease 2019 (COVID-19), which emerged in December 2019, has become a major worldwide public health problem. As of 1 April 1 2021 severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2, has rapidly spread to approximately 218 countries, causing 130 163 234 cases and 2 839 661 deaths [1].

Because of the high mortality rate of the COVID-19, it is important to identify prognostic factors and develop predictive models to estimate survival probability and better individualize treatments, especially for severe or critically ill COVID-19 patients. Demographic and clinical features have predictive power and studies have also demonstrated the significance of laboratory measurements, such as lactate dehydrogenase (LDH), hypersensitive c-reactive protein (Hs-CRP), aspartate aminotransferase (AST), creatine and D-dimer, in discriminating between COVID-19 patients who survived or deceased [2].

Clinical and laboratory biomarkers are usually measured at either regular or irregular intervals in COVID-19 patients during hospitalization [3]. Previous studies mainly focused on risk prediction based on laboratory measurements at a single time point (at baseline or the last measure before death or discharge from the hospital), ignoring the time course of biomarkers varying over time [3–5]. However, disease progression is a dynamic process. Static prediction based on a single time point may not provide sufficient information on how an individual patient's risk dynamically will update over time and how the risk is influenced by time-varying predictors [6]. Thus, it is attractive to make dynamic predictions by fully utilized information from longitudinal data measured at multiple time points, which may be beneficial for making timely and effective individual treatment recommendations [7, 8]. As an example, it is recognized that psychopathology is highly dynamic. Static prediction based on single baseline assessments may fail to produce accuracy and replicability predictions, whereas analytical methods built on the dynamic nature of psychopathology may be more powerful for predicting which individual may change from one clinical state to another and when this change will happen [9].

In this study, we aimed to develop a dynamic risk prediction model for COVID-19 prognosis based on the information of 1997 patients from Hubei Province, China, applying a random forest-based machine learning method and a joint modeling technique. The predictive model was firstly developed based on a publicly available dataset of 375 patients from Wuhan. In addition, our study characterized the time-dependent effect of laboratory biomarkers on prognosis. The model was further validated in two independent cohorts, including a cohort of 112 patients from Huangshi, Hubei and a cohort of 1527 patients from Wuhan Huoshenshan Hospital.

Methods

Data sources

For the discovery set, data of 375 COVID-19 patients from Tongji hospital collected between10 January and 18 February 2020 were obtained from a recently published literature [4]. Details about recruitment and inclusion/exclusion procedures have been described before [4]. Briefly, the dataset includes 197 general, 27 severe, and 151 critically ill COVID-19 patients. For the 375 patients, the age distribution (mean ± standard deviation) was 58.83 ± 16.46 years, 59.7% were male and 201 (53.6%) recovered from COVID-19 and were discharged from hospital. Period of follow-up was defined as the duration from hospital admission to death or discharge, and the maximal follow-up time was 35 days.

We validated the predictive model generated from the discovery stage in two independent cohorts. The first dataset includes 112 severe or critically ill COVID-19 patients recruited from three hospitals (Huangshi Central Hospital, Huangshi Hospital of Traditional Chinese Medicine and Daye People's Hospital) during 21 January–6 March 2020. All three hospitals are located in Huangshi City, Hubei Province, China. Detailed demographics and clinical characteristics, including initial symptoms, comorbidities and disease severity, were recorded at admission. Laboratory measurements, such as routine blood tests, lymphocyte subsets and inflammatory biomarkers, were obtained at admission and during hospitalization. More details about the study have been described previously [10]. The second validation set includes laboratory test results of 1527 severe or critically ill COVID-19 patients recruited between 4 February and 30 March 2020 from Wuhan Huoshenshan Hospital in China. Data on the clinical characteristics and laboratory findings of all patients were extracted from the hospital electronic medical records, and more information can be found in a previously published article [11].

The discovery dataset used in our study had been published and is publicly available, which was approved by the Tongji Hospital Ethics Committee [4]. As for the first validation dataset, the ethics committee of the hospitals (Huangshi Central Hospital, Huangshi Hospital of Traditional Chinese Medicine, and Daye People's Hospital) waived the written informed consent from patients with COVID-19, and all the procedures being performed were part of the routine care [10]. Written informed consent of Wuhan Huoshenshan Hospital was obtained from each patient, and the study was approved by the Medical Ethical Committee of Wuhan Huoshenshan Hospital and the Ethical Committee of Nanjing Medical University [11].

Statistical analysis

Descriptive statistics were obtained for all study variables. Continuous variables were summarized as means (± standard

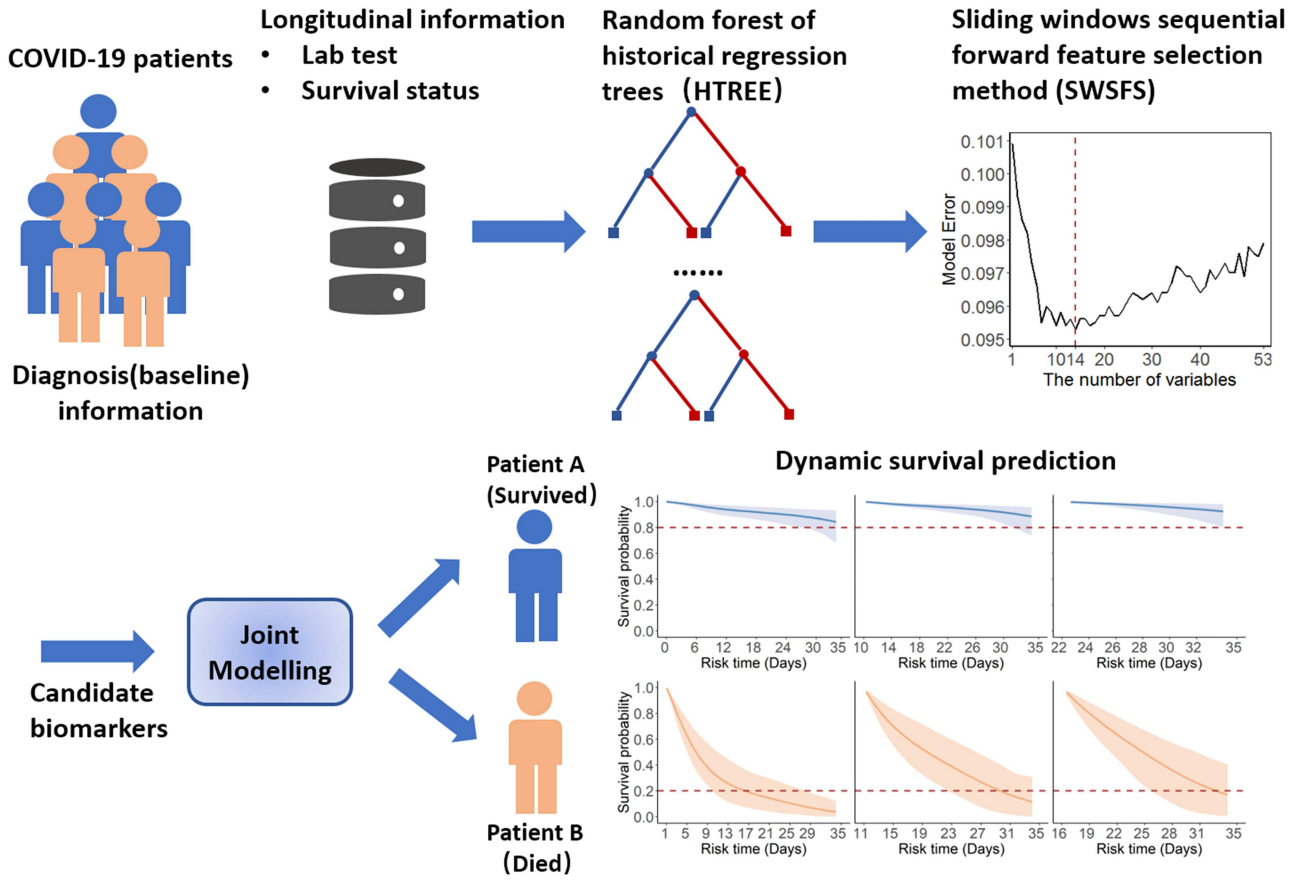


Figure 1. Strategy for incorporating longitudinal measurements to make dynamically predictions on individual prognosis for COVID-19 patients. First, baseline and laboratory tests information during hospitalization were used in HTREEs, and the SWSFS was used to determine the number of important biomarkers, identifying a set of important prognostic biomarkers. Then, joint modeling was applied to characterize the disease process and obtain dynamic predictions at the individual level in COVID-19 patients.

deviations), and the characteristics of patients who survived or died were compared using *t*-test or Mann–Whitney *U* test for continuous variables depending on the data distributions. Categorized variables were described by frequency (*n*) and proportion (%) and compared using chi-square test. As longitudinal biomarker measurements were only taken intermittently during hospitalization, missing values are unavoidable. Missing laboratory values were imputed using the "Mice" package in R [12]. All concentrations of laboratory measures were $\log_e$ transformed for further statistical analysis.

Model development

Development of the predictive model consisted of two main stages: (i) feature selection and (ii) dynamic characterization of the longitudinal process and time-to-event outcome. Each process was externally verified, and the analysis workflow is shown in Figure 1.

Feature selection

We applied historical regression trees (HTREEs) to screen for important biomarkers in COVID-19 patients by summarizing variable importance with longitudinal measurements using the R package "htree". HTREE is an extension of the standard random forest model appropriate for longitudinal data and a procedure for non-parametric estimates of how the response depends on all prior observations as well as that of any time-varying predictor variables. To evaluate stability of the model and reliability of the biomarkers, the prediction forest was evaluated by 3-fold cross-validation and was further assessed in two external validation datasets based on the candidate biomarkers.

In the discovery phase, all clinical characteristics during follow-up, including demographics, were evaluated in HTREE model. A sliding windows sequential forward feature selection method (SWSFS) was used to determine the number of "important" biomarkers. Briefly, the importance of each variable was evaluated by the average mean squared error which was first obtained by a HTREE analysis calculated via the *varimp_hrf* function. Variables were then included one by one to the HTREE model in the order of importance. We plotted the marginalized error, which measures the performance of each model consisting of different numbers of variables to identify a feature set with the lowest error rate. Finally, prognostic biomarkers and covariates in the selected feature set were used as HTREE input to construct a prediction forest model, which was further validated in two external validation datasets.

Joint model construction

We applied the joint modeling technique to fully use the repeated laboratory measurements and time-to-event data. Joint modeling is a method that can simultaneously analyze the longitudinally measured laboratory measurements and survival

outcome [13]. It consists of two linked sub-models: a mixed model specifying the time-course of biomarkers, and a survival model using the Cox proportional hazards model. For each of the prognostic biomarkers in the selected feature set, we developed a generalized linear mixed effect sub-model. The estimation of regression parameters within the multivariate joint model framework was performed using the R package "JMbayes" [13], which fits joint model under a Bayesian approach using the Markov Chain Monte Carlo algorithms [14]. For the survival sub-model, a Cox proportional hazard model with a penalized-spline-approximated baseline risk function was used [15] (Details about joint model can be found in the Supplementary Methods).

With the joint model, we were able to estimate the subject-specific marker trajectories and predict conditional survival probabilities for subject i, providing a set of longitudinal measurements$y_i(t)$[13]. The conditional probability of surviving to time u given that the patient has survived up to time t ($u > t$) was

$$\pi_i(u|t) = P\left(T_i^* \geq u | T_i^* > t, y_i(t)\right).$$

An estimate of $\pi_i(t)$ was computed by resampling from posterior distributions produced using the *survfitJM* function, assuming that the patient was event-free up to the time point of the last measurements [13, 14]. We calculated 5-day, 10-day and 15-day time-dependent areas under the receiver-operator characteristic curve (AUC) to evaluate the predictive accuracy of the joint model in both discovery and external validation datasets, as the 5th, 10th and 15th days after admission are critical time points at which the physician can take action to improve survival chances of the COVID-19 patients.

To investigate the potential nonlinear trend of biomarker effects varying over time, we allowed a time-varying coefficient to link longitudinal and survival processes using *P*-splines for the logarithm of the baseline hazard, with the idea to include interactions of biomarkers with an appropriate pre-defined time function (see Supplementary Methods) [16]. For the case of splines, baseline hazard was defined using *P*-splines with seven internal knots placed at equally spaced percentiles of the observed survival time t.

Statistical analyses were performed using R version 4.0.2 (The R Foundation of Statistical Computing). $P \leq 0.05$ was considered statistically significant unless otherwise specified.

Results

Characteristics of the study population

In the discovery phase, we used a previously described cohort that, after exclusion of 17 patients lacking more than 80% laboratory test data (see Supplementary Table 1), consisted of 358 COVID-19 patients enrolled from Tongji Hospital in Wuhan, Hubei Province, China [4]. The mean age of all 358 patients was 58.84 (16.51) years, and 210 (58.66%) were male. The median follow-up time since admission was 10 days, and 148 (41.34%) patients died in hospital. A comparison of baseline laboratory measures of patients who survived and those who died is presented in Table 1. In the validation phase, the first dataset consists of 112 patients from three hospitals in Huangshi City, Hubei Province, China. The mean age of these patients was 60.99 (14.87) years, and 73 (65.18%) were male. The median follow-up time since admission was 11 days, and 31 (27.68%) died (see Supplementary Table 2). For the second validation set including hospital records of 1527 severe or critically ill COVID-19 patients from Wuhan Huoshenshan Hospital in China, the mean age of these patients was 61.81 (14.13) years, and 775 (50.75%) were male. The median follow-up time since admission was 15 days, and 57 (3.73%) died (see Supplementary Table 3).

Feature selection

In the discovery dataset, two covariates (age and gender) and 53 laboratory test markers (see Supplementary Figure 1) with sufficient numbers of replicates and examined in at least 80% of COVID-19 patients were included in the HTREE model. SWSFS identified 14 top biomarkers: LDH, white blood cell (WBC) counts, neutrophil (NEU), mean platelet volume (MPV), creatinine, lymphocyte (%), Hs-CRP, prothrombin time (PT), red blood cell distribution width (RDW), urea, AST, glucose, monocytes (%) and procalcitonin (see Figure 1 and Supplementary Figure 2A). Three-fold cross-validation using an internal validation dataset further verified the importance of the candidate prognostic biomarkers. All 14 selected biomarkers were ranked in the top 25, and 7 were in the top 10 in the internal validation dataset, as shown in Supplementary Figure 2B. The mean AUC of this model was 98.32% [95% confidence interval (CI): 0.98–0.99] in the internal training dataset, and 96.49% (95% CI: 0.93–1.00) in the internal test dataset. Further, the predictive forest model was validated in two external validation datasets. The AUCs in the external validation datasets from Huangshi and Wuhan Huoshenshan Hospital reached 99.76% (95% CI: 0.99–1.00) and 97.63% (95% CI: 0.97–0.98), respectively (Figure 2A and B), showing the stability of the HTREE model and reliability of the predictive value of the selected biomarkers.

Joint model construction and assessment

With the linear mixed effect model in the joint modeling procedure, individualized prediction was made for longitudinal covariates using mean posterior fixed and random effects. Each participant would have predicted longitudinal measurements of biomarkers from hospital admission up to death or censoring. With the predicted biomarkers, we further made dynamic predictions on the risk of death for each individual since hospitalization. For example, the event-free probability curve of Patient A (survived) showed no apparent changes, whereas Patient B (died at the 32th day) showed considerable decline in event-free probability, indicating that Patient B had a higher risk of death, thus deserving frequent monitoring and close watching (see Figure 3). Dynamic prediction results for Patient B also indicated that long-term risk of death was higher in early visits but declined in later visits, which is in agreement with experiences obtained from medical practice. Predictive performance of the multivariate joint model is presented in Table 2. In particular, the time-dependent AUC is presented assuming a different prediction interval (5, 10 or 15 days), starting at a specific day (the 5th, 10th and 15th days after admission, respectively). The mean 5-day AUC was 87.61% (range: 83.43%–93.67%), mean 10-day AUC was 87.55% (range: 85.52%–88.95%) and mean 15-day AUC was 87.03% (range: 86.70%–87.29%) in the first validation dataset from Huangshi City. Meanwhile, the mean 5-day, 10-day and 15-AUC were 94.97% (range: 91.00%–97.86%), 95.78% (range: 93.58%–97.02%) and 94.63% (range: 93.48%–95.43%) in the second validation dataset from Wuhan Huoshenshan Hospital.

The predicted longitudinal measurements of patient A and patient B were characterized separately using smoothed curves

Table 1. Demographics, baseline clinical laboratory test and mortality outcomes collected from medical records in the discovery dataset

	Total ($n = 358$)	Survived ($n = 195$)	Dead ($n = 163$)	P-value
Age	58.84 ± 16.51	50.39 ± 15.00	68.94 ± 11.94	<0.0001[b]
Gender, *n* (%)				<0.0001[c]
Male	210 (58.66)	92 (43.80)	118 (56.19)	
Female	148 (41.34)	103 (69.59)	45 (30.40)	
Median follow-up (days)	10	14	6	<0.0001[b]
Laboratory tests (baseline)				
LDH, U/L	440.24 ± 308.77	270.23 ± 102.86	649.66 ± 347.17	<0.0001[b]
WBC, $10^{\wedge}$ 9/L	9.66 ± 14.19	7.75 ± 16.64	12 ± 10.03	<0.0001[b]
NEU, $10^{\wedge}$ 9/L	6.34 ± 5.18	3.53 ± 2.17	9.78 ± 5.69	<0.0001[b]
Hs-CRP, mg/L	76.32 ± 75.49	35.23 ± 43.45	128.31 ± 75.46	<0.0001[b]
MPV, fL	10.86 ± 0.94	10.63 ± 0.87	11.16 ± 0.94	<0.0001[a]
Lymphocyte, %	17.17 ± 12.65	24.85 ± 11.25	7.71 ± 6.28	<0.0001[b]
Monocyte, %	7.01 ± 4.27	8.56 ± 3.52	5.11 ± 4.36	<0.0001[b]
Procalcitonin, ng/ml	0.7 ± 3.36	0.09 ± 0.3	1.39 ± 4.85	<0.0001[b]
Creatinine, umol/L	98 ± 126.81	90.02 ± 152.6	107.83 ± 84.41	<0.0001[b]
PT, S	15.19 ± 5.65	13.82 ± 0.91	16.87 ± 8.09	<0.0001[b]
RDW, %	12.81 ± 1.61	12.33 ± 0.99	13.38 ± 1.97	<0.0001[b]
Urea, nmol/L	7.53 ± 6.77	4.88 ± 4.2	10.8 ± 7.84	<0.0001[b]
Glucose, mmol/L	8.30 ± 4.26	6.91 ± 2.93	9.94 ± 4.95	<0.0001[b]
AST, U/L	42.63 ± 61.57	28.08 ± 19.82	60.56 ± 86.14	<0.0001[b]

Note: Continuous variables are presented as mean ± standard deviations; categorical variables are presented as frequency and proportion *n* (%).
[a]P-value was derived from Student's *t*-test.
[b]P-value was derived from rank-sum test.
[c]P-value was derived from χ^2 test.

Table 2. Joint model results showing the 5-day, 10-day and 15-day areas under the receiver-operator characteristic curve (AUC) by different start follow-up time

Start day	At-risk	5-day AUC (%)	10-day AUC (%)	15-day AUC (%)
Discovery				
5	258	94.87	95.36	92.82
10	171	93.37	89.29	84.57
15	97	78.20	69.78	79.47
Mean		88.81	84.81	85.62
Validation[a]				
5	92	83.43	85.52	87.11
10	80	85.73	88.19	87.29
15	49	93.67	88.95	86.70
Mean		87.61	87.55	87.03
Validation[b]				
5	1431	91.00	93.58	93.48
10	1035	96.03	96.73	95.43
15	746	97.86	97.02	94.99
Mean		94.97	95.78	94.63

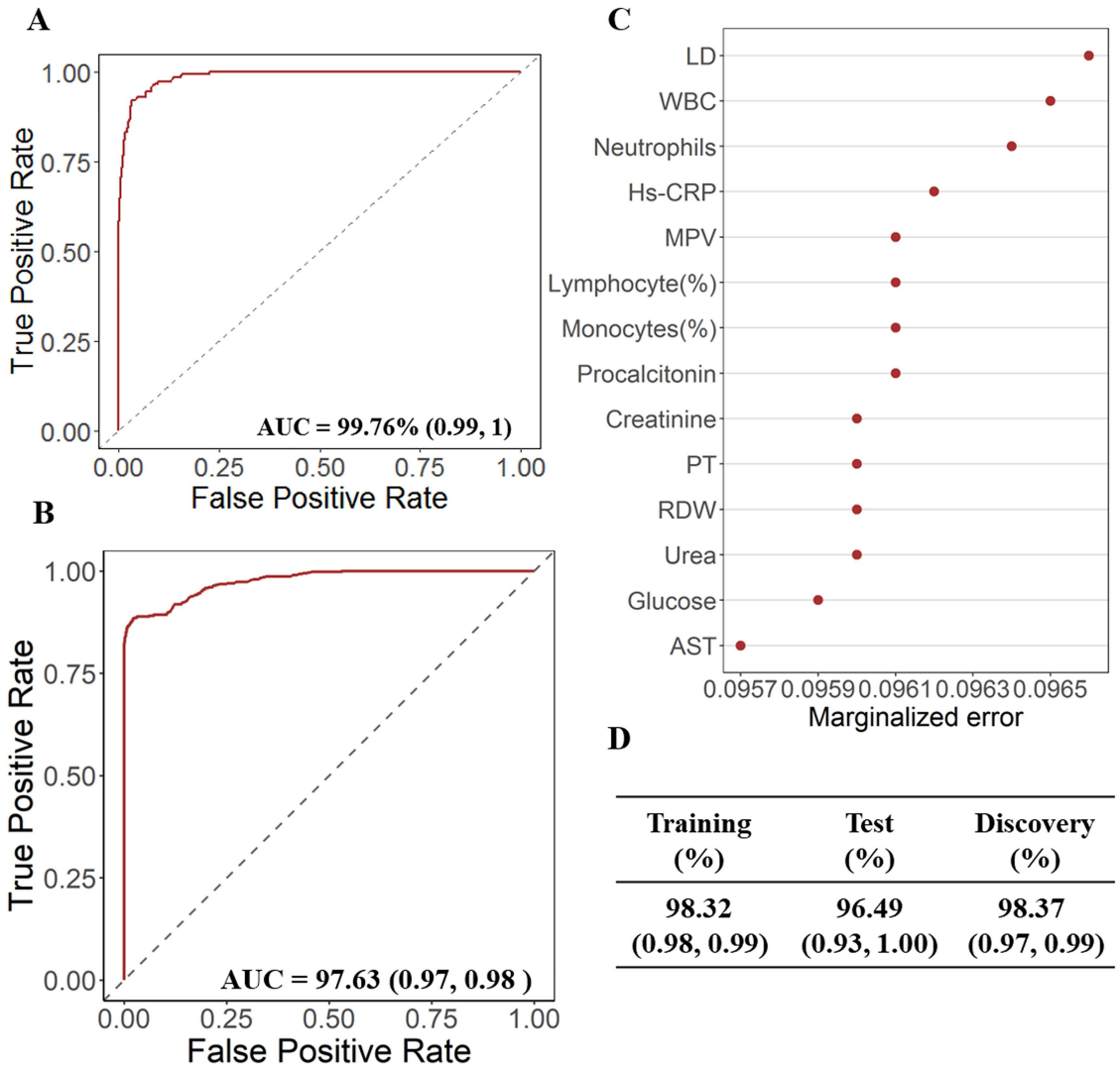
Note: AUC: the areas under the receiver-operator characteristic curve.
Validation [a]: the first validation dataset from Huangshi City.
Validation [b]: the second validation dataset from Wuhan Huoshenshan Hospital.
At-risk: the number of COVID-19 patients who were still at hospital.

(see Supplementary Figures 3). Biomarkers, excluding lymphocyte and monocytes, of Patient B were higher than those of Patient A. By fitting the changing trajectories of biomarkers over time for all patients, we found that patients who died and those who survived showed distinct patterns (see Figure 4 for the discovery dataset, Supplementary Figures 4 and 6 for the validation datasets).

Figure 5 shows how the effects of prognostic biomarkers on the event vary over time in the discovery dataset (see Supplementary Figures 5 and 7 for the validation datasets). The risk effects of NEU, Hs-CRP, MPV and urea gradually increased over time, whereas the effects of procalcitonin, PT and RDW declined. We observed relatively constant effects of LDH, WBC, creatinine, glucose and AST. Only lymphocyte and monocyte counts showed protective effects.

Web-based predictive tool

We developed an online tool to facilitate the application of our predictive model in practice (http://218.2.247.110:19040/COVID-19/Prediction). By inputting the values of prognostic factors of a COVID-19 patient, the tool would produce the conditional probability of death at specific time points.



Training (%)	Test (%)	Discovery (%)
98.32 (0.98, 0.99)	96.49 (0.93, 1.00)	98.37 (0.97, 0.99)

Figure 2. Performances of HTREE model and importance of 14 selected biomarkers. (**A**) Area under the receiver-operator characteristic curve (AUC) of the HTREE model including the 14 candidate biomarkers in the first validation dataset from Huangshi City. (**B**) Area under the receiver-operator characteristic curve (AUC) of the HTREE model including the 14 candidate biomarkers in the second validation dataset from Wuhan Huoshenshan Hospital. (**C**) Importance of the 14 selected biomarkers in the final model based on the discovery dataset. (**D**) Area under the receiver-operator characteristic curve (AUC) of the HTREE model including the 14 candidate biomarkers in discovery dataset and internal training and test sets using 3-fold cross-validation.

Discussion

Laboratory tests during the hospitalization of COVID-19 patients are usually repeated at regular or irregular intervals. It is of the clinicians' interest to make predictions of future outcomes based on all available information known up to a certain point of time. However, there is little research on dynamically prediction of COVID-19 prognosis based on repeated measurements and trajectories of laboratory biomarkers. In our study, we used the HTREE, an extension of random forests, to screen for biomarkers to predict the outcome of COVID-19 patients, thus boosting efficiency by incorporating information of longitudinal biomarker profiles [17]. The accuracy of our model was clinically satisfactory (98.37% of AUC in the discovery set, 99.76 and 97.63% of AUCs in two external validation datasets). We also used multivariate joint model to characterize the time-to-event process and obtain dynamic survival predictions at the individual level, providing useful and practical information to support individualized decisions for the treatment on COVID-19 patients. Also, time-varying associations between survival status and longitudinal biomarkers may be helpful for choosing timely treatment strategy that could improve prognosis.

We identified 14 important biomarkers among 53 clinical features, of which 12 were risk factors associated with the mortality of COVID-19 patients. LDH had the highest importance for predicting COVID-19 patient survival, consistent with previous studies [4, 18]. Decreased LDH level was related to the elimination of viral messenger RNA and correlated with shorter hospital stay, indicating better COVID-19 prognosis [19].

Like LDH, most of the remaining identified biomarkers (WBC, NEU, Hs-CRP, MPV, procalcitonin, creatinine, urea, AST, PT, RDW and glucose levels) are associated with poor COVID-19 prognosis. Leukocytosis and neutrophilia are hallmarks of acute infection [18, 20–22]. Hs-CRP and procalcitonin are commonly used inflammatory markers in the clinic, the increase of which may indicate poor prognosis in COVID-19 patients [23, 24]. It has been reported that high MPV level, related to thrombotic

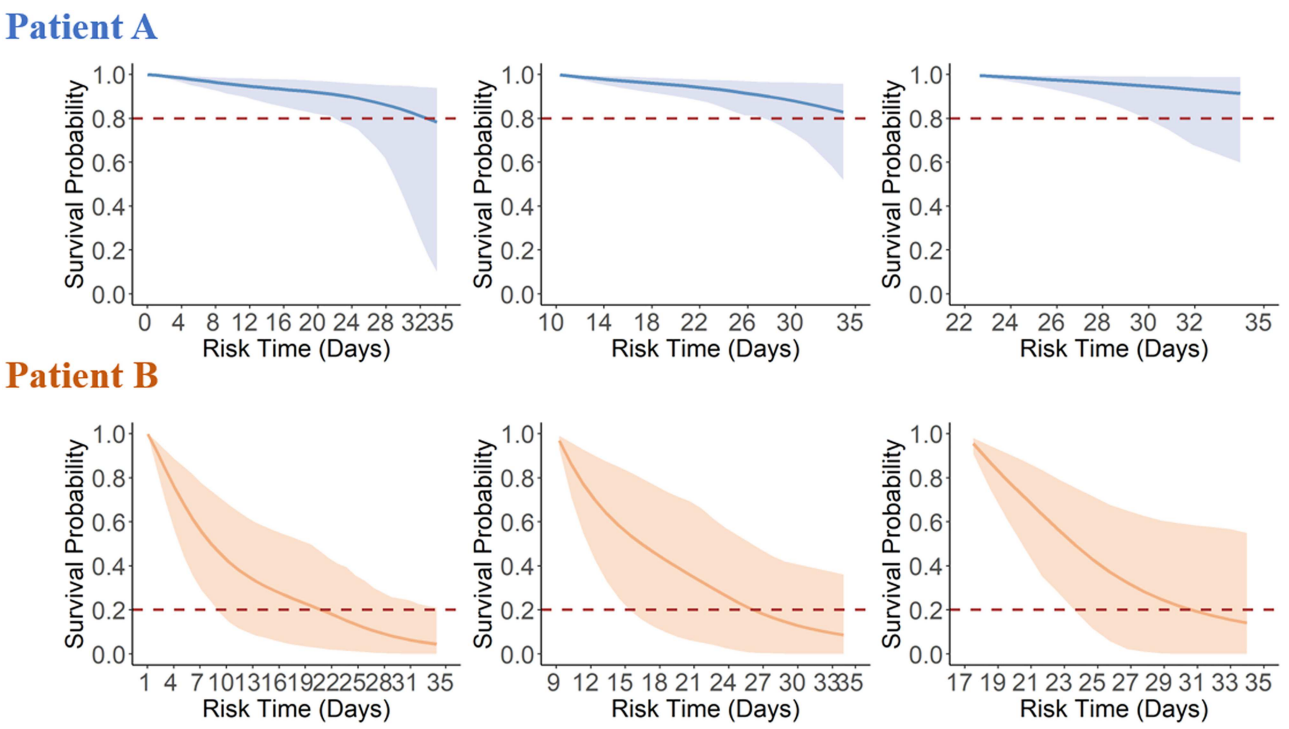


Figure 3. Dynamic predictions on the risk of death for Patient A and Patient B since hospitalization in the discovery dataset. Patient A had a total of seven laboratory test time windows and was discharged at the 35th day. The three graphs show survival probabilities of patient A at the follow-up visit 1th (day 0), visit 5th (day 10), and visit 7th (day 22). Patient B had a total of nine laboratory test time windows and was dead at the 32th days. The graphs show survival probabilities of patient B at follow-up visit 1th (day 1), visit 5th (day 11) and visit 8th (day 16).

events in COVID-19, is an independent risk factor for disease progression [25, 26]. Further, concentrations of creatinine, urea and AST, related to myocardial injury, were markedly higher in COVID-19 patients who died than in those patients who survived [23, 27, 28]. Elevated procalcitonin and PT levels were reported to indicate the degree of severity for COVID-19 cases [29, 30]. RDW is a readily available laboratory measure reflecting the extent of anisocytosis, which is a proposed part of the routine panel of laboratory tests offered for monitoring COVID-19 patients [31, 32]. Glucose level is an independent risk factor for severe or critical COVID-19, and well-controlled blood glucose correlates with improved prognosis [33]. On the other hand, monocytes and lymphocyte, which play central roles in the maintenance of immune system function of humans and in protecting the body from virus infections, showed protective effects in our research [34, 35]. In this study, the numbers of monocytes and lymphocyte of the patients who survived were significantly higher than those who died. Moreover, lower lymphocyte levels, platelet counts, higher blood urea and neutrophils have been reported to be associated with neurological disorders which has been a public concern for COVID-19 survivors [36–38].

All beneficial and risk biomarkers identified in this study have been previously reported to be associated with the prognosis of COVID-19 patients. Our study extends the findings to show how the time-varying patterns of biomarkers can dynamically predict individual outcome, as well as inform timely and precise treatment recommendations. As an example, the clinician may need to pay attention to LDH changes during the whole course of treatment for its constant and strong risk effect, whereas it may be significant to prevent high level of NEU in the late stage of treatment. Our results provide the possibility for clinicians to adapt appropriate treatment timely by monitoring the change of easily available biomarkers [39].

The analytical strategy we applied in this study, which is a combination of machine learning method and joint modeling technique, provides insights on the comprehensive analysis on clinical data arising from personalized medicine and real-world circumstances, in particular longitudinal measurements provided by electronic health records [40]. Fully usage of longitudinal measurements can inform the probability of an outcome of interest occurring at a future time [41]. Specially, joint modeling is able to account for measurement errors of the biomarkers and model biomarkers' trajectories over time. This strategy also enables the identification of potential interactions between nonlinear independent effects and time.

Our study has several strengths. Firstly, our study includes 1997 COVID-19 patients, which is one of the largest studies on COVID-19 prognosis. Secondly, we used an advanced machine learning technique, HTREEs, to identify important biomarkers. This method makes full use of longitudinal biomarkers and inherits the advantages of random forest algorithm, ensuring good stability and accuracy for further prediction [42, 43]. We also adopted multivariate joint model to characterize the time-to-event process, obtain dynamic predictions at the individual level and describe time-varying associations between the longitudinal biomarkers and the event. This facilitates the identification of critical time points and can alert the clinician when to apply patient-tailored therapies [44]. The performance of our final predictive model was also externally validated, achieving satisfactory AUC results in two independent datasets. Furthermore, we developed an online tool for clinicians to facilitate the application of our model.

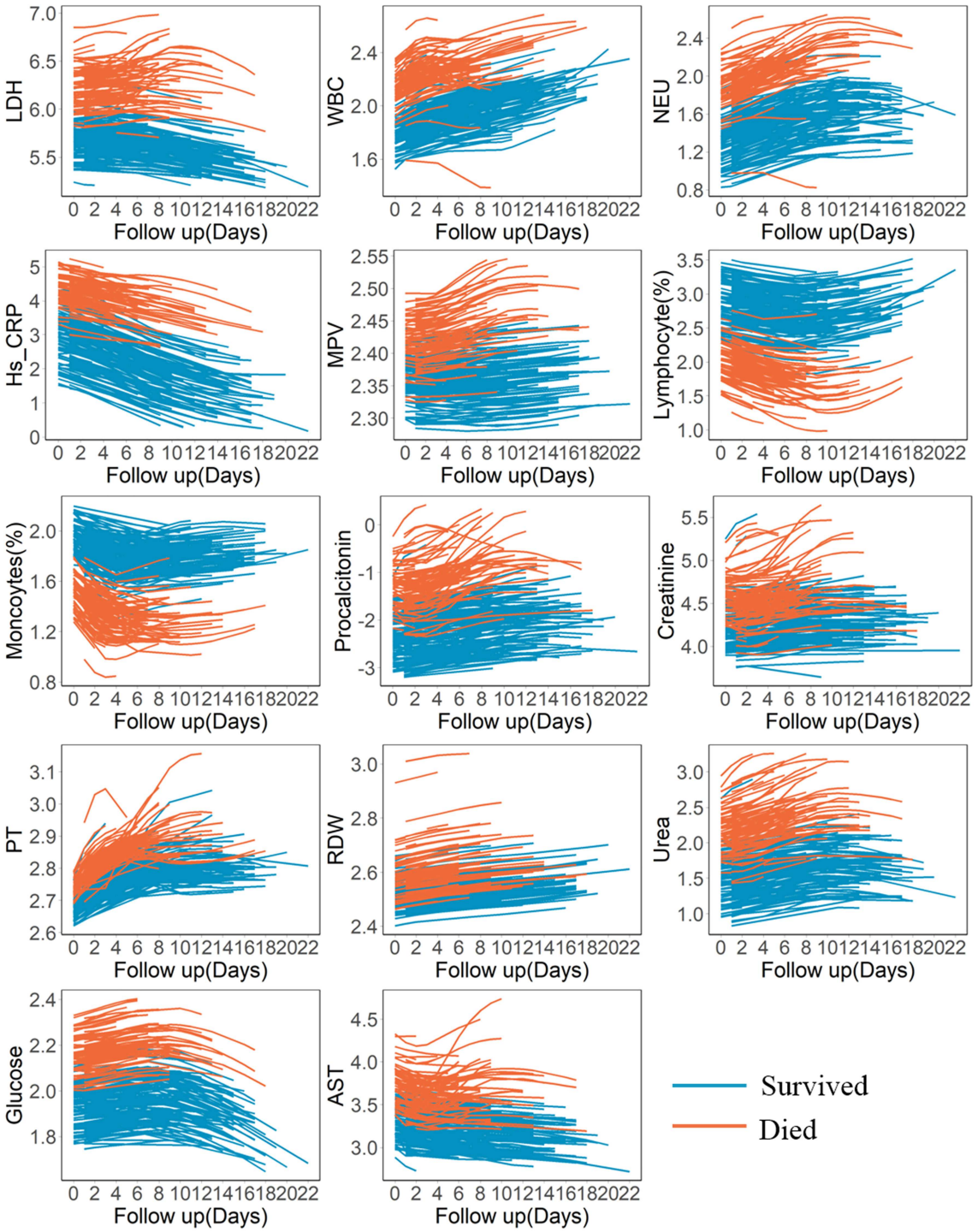


Figure 4. Fitting trajectory patterns of longitudinal biomarkers for patients who survived or deceased in the discovery dataset. Lines represent averaged trajectories of patients who survived (blue) or deceased (red) during hospitalization using natural cubic splines with two degrees of freedom.

However, our study also has several limitations. First, some patients had missing values for laboratory tests. Second, the mechanism of time-varying dynamic effects requires further investigation from a clinical perspective. Third, the prediction model of this study was trained and validated using Chinese population. Therefore, caution should be exercised when generalizing out findings to other race populations.

In conclusion, our study identified important biomarkers for early prediction on the outcome of COVID-19 patients by using a novel strategy suitable for dynamic risk prediction incorporat-

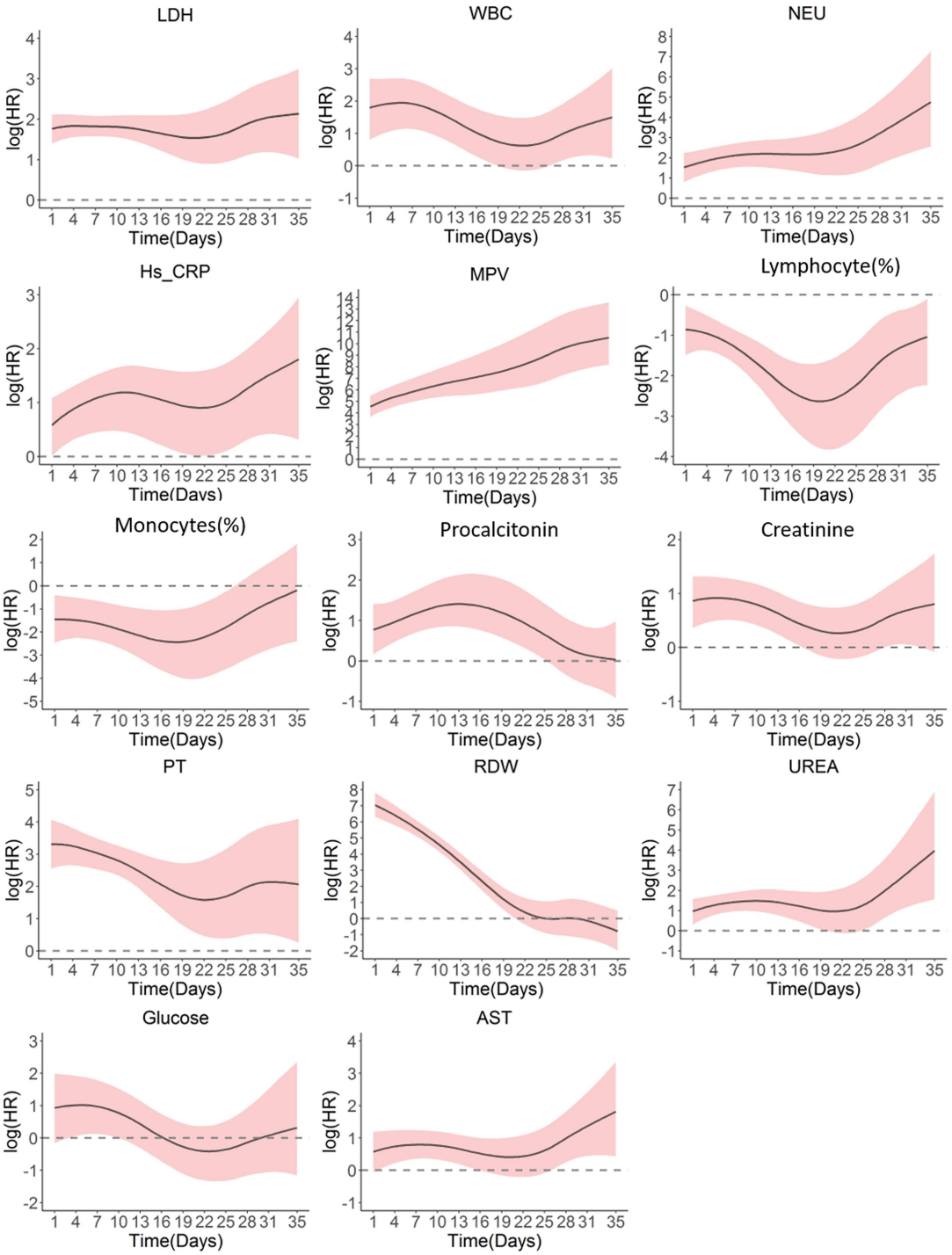


Figure 5. Time-varying effects of the biomarkers in the discovery dataset.

ing longitudinal laboratory measurements. The well-performing predictive model, with its accompanying online web application, is of great importance for the clinician to identify patients under high risk of death, as well as to characterize how the effects of biomarkers vary over time.

Key Points

- Longitudinal data could provide more information on disease progression and the possibility of the dynamic prediction on probabilities of survival over time in COVID-19 patients.

- Fourteen important biomarkers, including lactate dehydrogenase, white blood cell counts, neutrophil, mean platelet volume, creatinine, lymphocyte (%), hypersensitive c-reactive protein, prothrombin time, red blood cell distribution width, urea, aspartate aminotransferase, glucose, monocytes (%) and procalcitonin, were identified to be associated with the mortality of COVID-19 patients.
- This research characterized the time-to-event process, obtained dynamic predictions at the individual level and demonstrated the time-varying associations between the candidate longitudinal biomarkers and the mortality in COVID-19 patients.
- Our study provides a novel strategy suitable for dynamic risk prediction incorporating repeated measurements, which is very practical for clinical research.

Supplementary Data

Supplementary data are available online at https://academic.oup.com/bib.

Author contributions

X.C., W.G., D.Y., F.C., X.L. and Y.Z. contributed to the study design. X.C., W.G., J. L., Y.D., Z.Y., M.Z., Q.W. and F.S. contributed to data collection. X.C., W.G. and D.Y. performed statistical analyses and interpretation and drafted the manuscript. Z.Y. developed the online application. J.L., M.Z., F.S., Y.W., R.Z., T.L., Q.W., F.C., X.L. and Y.Z. revised the manuscript. All authors approved the final version. Financial support and study supervision were provided by Y.Z., F.C., Y.W., W.G. and X.L..

Data Availability

In discovery phase, data are available from the published literature, which has been published online [4]. The relevant validation datasets are available from the corresponding author upon reasonable request.

Consent for publication

All authors have reviewed the manuscript and approved the final draft for publication.

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