MyJA .pub

© Malaysian Journal of Anaesthesiology 2023; 2:134-144
Original article
https://doi.org/10.35119/myja.v2i2.56

Derivation of a multi-biomarker model for predicting mortality in hospitalised COVID-19 patients

Nur Izyan Izzati **Sathari**, Priyaneka **Baskaran**, Laila **Ab Mukmin**, Mohd Zulfakar **Mazlan**, Wan Fadzlina **Wan Muhd Shukeri**

Department of Anaesthesiology and Intensive Care, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Abstract

Introduction: This study aimed to derive and assess the performance of a multi-biomarker model from a combination of basic laboratory biomarkers in predicting mortality of hospitalized COVID-19 patients.

Methods: This was a cross-sectional study conducted in a university-affiliated hospital in Malaysia. Data of confirmed COVID-19 patients who were admitted from January 2020 to August 2021 were retrieved including their admission C-reactive protein (CRP), lactate dehydrogenase (LDH), and neutrophil-lymphocyte ratio (NLR). Patients were classified as non-survivors or survivors according to their hospital mortality status. Multi-variable logistic regression analysis was used to derive the multi-biomarker model.

Results: A total of 188 confirmed COVID-19 patients were analysed, of which 46 (23%) died in the hospital. Their mean age was 52 (SD 17) years, 104 (52%) were males, 114 (57%) had severe COVID-19 pneumonia, with mean APACHE II score of 14 (SD 10). On admission, those who died had higher median levels of CRP 96.0 (IQR 39.8–182.0) vs 23.0 (IQR 0–67.0 mg/L, $p < 0.001$), of LDH 973.0 (IQR 706.5–1520.0) vs 515.1 (408.8–738.8 IU/L, $p < 0.001$), and of NLR 10.1 (IQR 5.5–23.6) vs 2.8 (IQR 1.5–5.9, $p < 0.001$).

Correspondence: Assoc. Prof. Dr. Wan Fadzlina Wan Muhd Shukeri, PhD, Department of Anaesthesiology and Intensive Care, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
E-mail: wfadzlina@usm.my

The multi-biomarker model had a higher area under the curve (0.866, 95% CI 807-0.925) compared to its constituent individual biomarkers. At its optimal cutoff, this model had 78.9% sensitivity and 76.5% specificity for mortality prediction.

Conclusion: A multi-biomarker model of CRP, LDH, and NLR predicted in-hospital mortality with a very good performance in our hospitalised COVID-19 patients.

Keywords: biomarkers, COVID-19, mortality

Introduction

Being declared a global pandemic in March 2020, coronavirus disease 2019 (COVID-19) has spread worldwide and costs millions of lives. COVID-19 has variable clinical presentation, from asymptomatic or milder symptoms such as cough, fever, sore throat, myalgia, and headache, to more severe manifestations such as confusion, chest pain, hypoxemia, pneumonia, and other complications that require mechanical ventilation and intensive care unit (ICU) admission.[1] Severe COVID-19 has a high mortality risk; therefore, it is important to effectively predict which of these patients are more likely to die in order to provide early and timely intervention.

Many studies have reported that using biomarkers can help to predict the outcome of hospitalised COVID-19 patients. Numerous biomarkers are used for prognostication of COVID-19. Examples of such biomarkers are C-reactive protein (CRP), lactate dehydrogenase (LDH), and neutrophil-to-lymphocyte ratio (NLR). CRP is a non-specific acute phase reactant elevated in infection or inflammation; higher levels indicate more severe infection and have been used as an indicator of COVID-19 disease severity.[2,3] LDH is one of the enzymes of the glycolytic pathway that catalyses the conversion of pyruvate to lactate; elevated LDH levels have been shown to be associated with more severe disease and increased mortality in multiple diseases, including severe COVID-19.[4,5] NLR, obtained by dividing the absolute neutrophil count by the absolute lymphocyte count, has great value in indicating a patient's overall systemic inflammatory status. Its changes can not only reflect the role of neutrophils in infection, but also reflect the changes of lymphocytes. According to recent studies, NLR has some predictive value in predicting the severity and mortality in patients with COVID-19.[6]

While there are many more biomarkers being evaluated for the prognostication of COVID-19, it is unlikely that a single biomarker approach would be able to reflect the various host responses to the infection. Clinicians and researchers have been

making efforts to understand COVID-19, but knowledge of its pathogenesis is still not fully understood. A multi-biomarker approach, one which requires several biomarkers being measured and jointly interpreted, could be a superior alternative to assess prognosis in COVID-19 pneumonia. This is because such an approach would be more likely to reflect the various host responses to the COVID-19 infection.

To date, there have been limited studies available regarding the prognostic use of a multi-biomarker approach in the COVID-19 literature. Studies that measure several biomarkers that are interpreted separately do not constitute a multi-biomarker approach. Derivation of a new multi-biomarker model, perhaps one using inexpensive and routinely available biomarkers, may prove to be useful in predicting the in-hospital mortality of hospitalised COVID-19 patients. The aim of this study was to derive and assess the performance of a multi-biomarker model from the combination of basic laboratory biomarkers, namely CRP, LDH and NLR, in predicting mortality of hospitalized COVID-19 patients.

Methods

Study design and participants

This cross-sectional study was conducted after receiving ethical approval from our institution's Human Research and Ethics Committee (Study protocol code: 21100653). Request of the waiver of written informed consents was granted given that the study involved retrospective chart review of the subjects. The inclusion criteria for the study were adult patients (aged 18 years or above) with confirmed COVID-19 patients who were admitted to our institution between January 2020 to August 2021. Patients with incomplete data of biomarkers of interest were excluded.

Data collection methods

In the included patients, relevant demographic, and clinical data were retrieved from their medical record. The data included their age, gender, ethnicity, comorbidities, stage, and severity of COVID-19 on admission, general and specific treatments received in the first 24 hours of admission, and biochemistry profiles. In addition, CRP, LDH, and NLR, measured in the first 24 hours of hospital admission were recorded retrospectively from the hospital computerised database. Of note, NLR was manually calculated as absolute neutrophil count divided by the absolute leukocyte count.

Statistical analysis

Data analysis in this study was performed using IBM Statistical Package for the Social Sciences (SPSS) software. All categorical variables were presented as frequency and proportion, while all numerical variables were presented as mean and standard deviation, or as median and interquartile range, depending on their normality of distribution.

Comparisons of categorical variables between two groups (survivors and non-survivors) were made using the Chi-square test or the Fisher's exact test, as appropriate. Comparisons of numerical variables between the two groups (survivors and non-survivors), including the biomarkers, were made using the independent T-test or the Mann-Whitney U-test, as appropriate.

To derive the multi-biomarker model, we used binary logistic regression analysis by including all the three biomarkers *i.e.*, CRP, LDH and NLR, as covariates, and in-hospital mortality as the dependent variable, employing the enter method. Using the generated coefficients in the model equation, the probabilities of the event, *i.e.*, in-hospital mortality, were reported. This had a value of 0 to 1. The Hosmer-and-Lemeshow goodness-of-fit test was performed to determine the calibration of the model, in which $p > 0.05$ indicates that the model is well-calibrated.

The prognostic performance of the multi-biomarker model and its constituent individual biomarkers were assessed by the area under the curve (AUC). The AUC ranges from 0.5 (no discrimination) to 1 (perfect discrimination). Clinical validity is assumed at an AUC of more than 0.7. The sensitivity and specificity of the biomarkers at the optical cutoff were calculated; optimal cut-off was defined as the measured quantity which maximized sensitivity and specificity. For all analyses, differences were considered statistically significant at $p < 0.05$.

Sample size calculations

We wished to show that the AUC of 0.814 for the multi-biomarker model, based on a previous study, is significantly different from the null hypothesis value of 0.5.[7] Using a ratio of the sample between negative and positive cases of 113:46, significance at 0.05, and power of 0.8, we needed to study 23 survivors and 9 non-survivors, giving a total of at least 32 patients with COVID-19 to be studied.

Results

Throughout the 20-month study period, a total of 199 patients were screened for eligibility. Eleven (5.5%) of these 199 patients were excluded from the analysis due to incomplete data of the studied biomarkers. As such, we were left with 188 patients to be analysed, of which the outcome of in-hospital mortality was reached in 46 (23.0%) patients. These patients were classified as non-survivors in this analysis.

Table 1. Demographics, comorbidities, disease characteristics, and biochemical profiles

Variable		**Survivors (n = 142)**		**Non-survivors (n = 46)**		***p*-value**
Demographics						
Age		51	(31)	65	(13)	< 0.001
Gender	Male	75	51.0%	24	58.5%	0.394
	Female	72	49.0%	17	41.5%	
Ethnicity	Malay	137	93.2%	39	95.1%	0.656
	Non-Malay	10	6.8%	2	4.9%	
Comorbidities						
Comorbidities	No	88	59.9%	17	41.5%	0.036
	Yes	59	40.1%	24	58.5%	
Disease characteristics						
Stage	Stage I-III	82	55.8%	0	0.0%	< 0.001
	Stage IV-V	65	44.2%	41	100.0%	
Severity	Non-severe	80	54.4%	1	2.4%	< 0.001
	Severe	67	45.6%	40	97.6%	
Biochemical profiles						
WBC		5.90	3.84	7.16	7.79	0.013^
ANC		3.95	3.96	7.41	8.68	<0.001^
ALC		1.29	0.99	0.60	0.57	<0.001^

WBC: white blood cell; ANC: absolute neutrophil count; ALC: absolute lymphocyte count

Baseline characteristics

COVID-19 patients who died during admission were significantly older than those who went on to survive (Table 1). A significantly higher proportion of the non-survivors had baseline comorbidities compared to the survivors (Table 1). In terms of the COVID-19 disease characteristics, a higher proportion of the non-survivors presented at stage IV-V and with higher severity compared to the survivors (Table 1). In terms of biochemical profiles, the white blood cell count and absolute neutrophil count were significantly higher, while absolute lymphocyte count was significantly lower in the non-survivors compared to the survivors (Table 1).

For descriptive purposes, a significantly higher proportion of the non-survivors received general treatment of intubation, high-flow nasal cannula, non-invasive ventilation, do-not-resuscitate status, and ICU care in the first 24 hours of hospitalization, compared to the survivors (Table 2). Also, a significantly higher proportion of the non-survivors were given specific treatment of antibiotics, antiviral, steroid, and anticoagulant (Table 2).

Biomarker profiles

The median and interquartile ranges are shown for each of the three biomarkers as stratified by their in-hospital mortality status (Table 3). As a summary measure of predictive accuracy, we determined the AUC and the ideal cut-off values for the ability of each biomarker to classify patients with in-hospital mortality. As shown in Table 3, all biomarkers are significantly higher in the non-survivors compared to the survivors and are clinically valid in predicting in-hospital mortality, as indicated by the AUC > 0.7.

Derivation of the model

We then used binary logistic regression to combine all biomarkers to determine the combination's association with in-hospital mortality. The resulting logistic regression equation is logit (probability of in-hospital mortality) = -4.215 + (0.009 x CRP) + (0.003 x LDH) + (0.008 x NLR). The Hosmer-and-Lemeshow test was not significant ($p = 0.668$), indicating adequate calibration of the model.

Prognostic performance of the model

We found that the AUC of the combined biomarkers was 0.866 (95% CI 0.807–0.925, $p < 0.001$), which suggested a very good model discrimination. Of note, the AUC of the combined biomarkers is higher than its constituent individual biomarkers (Fig. 1), indicating better performance of the multi-biomarker approach in predicting mortality in COVID-19 patients compared to the single biomarker approach.

Table 2. General and specific treatments in the first 24 hours

Variable		**Survivors (n = 142)**		**Non-survivors (n = 46)**		***p*-value**
General treatment in the first 24 hours						
Intubated	No	146	99.3%	21	51.2%	< 0.001
	Yes	1	0.7%	20	48.8%	
HFNC	No	126	85.7%	26	63.4%	0.001
	Yes	21	14.3%	15	36.6%	
NIV	No	139	94.6%	31	75.6%	0.001
	Yes	8	5.4%	10	24.4%	
MV	No	145	98.6%	22	53.7%	< 0.001
	Yes	2	1.4%	19	46.3%	
DNR	No	141	95.9%	11	26.8%	< 0.001
	Yes	6	4.1%	30	73.2%	
ICU	No	139	94.6%	20	48.8%	< 0.001
	Yes	8	5.4%	21	51.2%	
Specific treatment in the first 24 hours						
Antiviral	No	128	87.1%	30	73.2%	0.032
	Favipiravir	19	12.9%	11	26.8%	
Antibiotic	No	60	40.8%	1	2.4%	< 0.001
	Yes	87	59.2%	40	97.6%	
Steroid	No	65	44.2%	0	0.0%	< 0.001
	Yes	82	55.8%	41	100.0%	
Anticoagulant	No	73	49.7%	2	4.9%	< 0.001
	Yes	74	50.3%	39	95.1%	

HFNC: high-flow nasal cannula; MV: mechanical ventilation; DNR: do not resuscitate; NIV: non-invasive ventilation

Table 3. Biomarker profiles

Biomarker	Survivors (n = 142)		Non-survivors (n = 46)		p-value*	AUC	Cutoff
	Median	IQR	Median	IQR			
LDH (U/L)	**511**	**329**	**973**	**814**	**< 0.001**	**.824**	**380**
CRP (mg/L)	18.0	65.0	96.0	148	< 0.001	.760	25
NLR	2.76	4.43	9.97	19.22	< 0.001	.830	1.83

LDH: lactate dehydrogenase; CRP: C-reactive protein; NLR: neutrophil-to-lymphocyte ratio, AUC: area under the curve

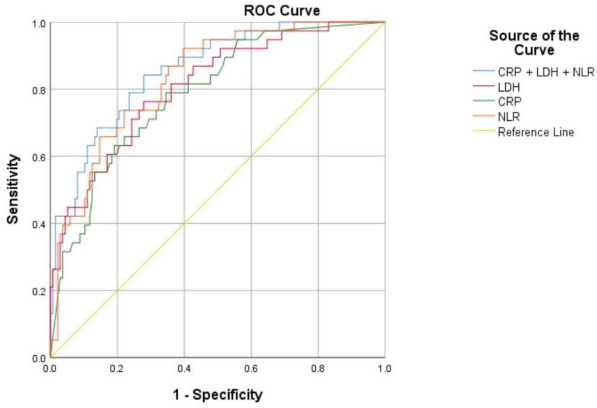

Fig. 1. Receiver-operating characteristic curves of the multi-biomarker model compared to its constituent individual biomarkers for their mortality predictive performance. CRP: C-reactive protein; LDH: lactate dehydrogenase; NLR: neutrophil-to-lymphocyte ratio.

Discussion

In this cross-sectional study, we assembled a cohort of 188 hospitalised COVID-19 patients and studied three biomarkers on their admission with the overall goal of deriving a multi-biomarker model that would allow discrimination of those who are at increased risk of in-hospital mortality. A multi-biomarker model using baseline CRP, LDH, and NLR predicted in-hospital mortality with a very good performance in our hospitalised COVID-19 patients. Of note, the multi-biomarker outperformed its constituent individual biomarkers in predicting in-hospital mortality in our COVID-19 cohort.

We believe that our results are novel with respect to the combined use of CRP, LDH, and NLR, each of which represents different aspects of the host inflammatory response to COVID-19 infection. At present, the pathophysiological process of COVID-19 infection is not fully understood. However, it is likely that the disease represents a complex interplay between various inflammatory responses rather than a singular type of response to the infection. As such, a multi-biomarker model that corresponds to various possible inflammatory processes is a logical approach in the prognostication of the disease.

Emerging evidence suggests that the multi-biomarker approach shows promising results in predicting the outcome of COVID-19. For example, Smilowitz *et al.* demonstrated that combination of three biomarkers (cardiac troponin, d-dimer, and CRP) yielded an improvement in the AUC of a clinical model from 0.765 to 0.879.[8] In another study, Zhou *et al.* found that combination of interleukin-6, neutrophil count, and natural killer cells had a high prediction accuracy for mortality in the training data as well as in the independent data of hospitalised COVID-19 patients.[9] Another study by Wang *et al.* using a multiplexed proteomics assay of up to 50 peptides derived from 30 known and newly introduced COVID-19-related protein markers predicted death with an accuracy of 0.76, which outperformed compound clinical risk assessment such as the Sequential Organ Failure Assessment score and the Acute Physiological and Chronic Health Evaluation II score.[10]

The strength of this study is the ability of our multi-biomarker model to predict an objective, rather than a subjective endpoint, namely in-hospital mortality, in the patients who would not necessarily be regarded as high-risk, *i.e.*, patients with Stage I to III COVID-19. All three biomarkers used are routinely available across many centres, and therefore usage of the multi-biomarker model is feasible to be applied in daily clinical settings. Also, all blood samples were collected within 24 hours of hospital admission, making the study reproducible while generating a prediction system that can be used from as early as the first day of hospital admission.

Although our results are encouraging, this study has several limitations. First, due to the retrospective nature of our study design, other biomarkers that could have performed well were not analysed, including other acute phase reactants such as albumin and ferritin. Second, the multi-biomarker model that we derived predicted our single-centre data set, but whether it is generalisable to external population is unknown. Third, our multi-biomarker model will require validation on an independent data set, ideally in a prospective study. Last, because this study used a convenience sampling method, selection bias may have led to a non-representative population. Therefore, further research is warranted to validate the clinical utility of our multi-biomarker model in the prediction of mortality in COVID-19.

Conclusion

Our study suggests that CRP, LDH, and NLR have positive associations with mortality in COVID-19. A multi-biomarker model using a combination of these individual biomarkers adequately predicted in-hospital mortality and outperformed its constituent individual biomarkers in our hospitalised COVID-19 patients. As such, a simple multi-biomarker approach using basic laboratory parameters of CRP, L and NLR is a potentially reliable to aid in the prognostic of COVID-19 patients, although this requires further validation in a prospective study.

Declarations

Ethics approval and consent to participate

This cross-sectional study was conducted after receiving ethical approval from Universiti Sains Malaysia Human Research and Ethics Committee (USM/JEPeM/21100653). Request of the waiver of written informed consents was granted given that the study involved retrospective chart review of the subjects.

Competing interests

Dr. Wan Fadzlina Wan Muhd Shukeri serves as Section Editor for Malaysian Journal of Anaesthesiology. She has not been involved in any part of the publication process prior to manuscript acceptance; peer review for this journal is double blind. The remaining authors have no competing interests to declare.

Funding
None to declare.

Acknowledgements
None to declare.

References

1. The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) — China, 2020. China CDC Wkly. 2020;2(8):113–22.
2. Yitbarek GY, Walle Ayehu G, Asnakew S, et al. The role of C-reactive protein in predicting the severity of COVID-19 disease: A systematic review. SAGE Open Med. 2021;9:20503121211050755. doi:10.1177/20503121211050755
3. Stringer D, Braude P, Myint PK, et al. The role of C-reactive protein as a prognostic marker in COVID-19. Int J Epidemiol. 2021;50(2):420-429. https://doi.org/10.1093/ije/dyab012
4. Huang Y, Guo L, Chen J, et al. Serum lactate dehydrogenase level as a prognostic factor for COVID-19: a retrospective study based on a large sample size. Front Med (Lausanne). 2022;8:671667. https://doi.org/10.3389/fmed.2021.671667
5. Szarpak L, Ruetzler K, Safiejko K, et al. Lactate dehydrogenase level as a COVID-19 severity marker. Am J Emerg Med. 2021;45:638-639. https://doi.org/10.1016/j.ajem.2020.11.025
6. Liu Y, Du X, Chen J, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect. 2020;81(1):e6-e12. https://doi.org/10.1016/j.jinf.2020.04.002
7. Shukeri WFWM, Ralib AM, Abdulah NZ, Mat-Nor MB. Sepsis mortality score for the prediction of mortality in septic patients. J Crit Care. 2018;43:163-168. https://doi.org/10.1016/j.jcrc.2017.09.009
8. Smilowitz NR, Nguy V, Yindalon A, et al. Multiple biomarker approach to risk stratification in COVID-19. Circulation. 2021;143(13):1338–40.
9. Zhou Z, Li Y, Ma Y, Zhang H, Deng Y, Zhu Z. Multi-biomarker is an early-stage predictor for progression of Coronavirus disease 2019 (COVID-19) infection. Int J Med Sci. 2021;18(13):2789-2798. https://doi.org/10.7150/ijms.58742
10. Wang Z, Cryar A, Lemke O, et al. A multiplex protein panel assay for severity prediction and outcome prognosis in patients with COVID-19: An observational multi-cohort study. EClinicalMedicine. 2022;49:101495. https://doi.org/10.1016/j.eclinm.2022.101495