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Original Article

Haematological and radiological-based prognostic markers of COVID-19



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ABSTRACT

Background: Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has emerged in 2019 and caused a global pandemic in 2020, manifesting in the coronavirus disease 2019 (COVID-19). The majority of patients exhibit a mild form of the disease with no major complications; however, moderate to severe and fatal cases are of public health concerns. Predicting the potential prognosis of COVID-19 could assist healthcare workers in managing cases and controlling the pandemic in an effective way. Therefore, the objectives of the study were to search for biomarkers associated with COVID-19 mortality and predictors of the overall survival (OS).

Methods: Here, clinical data of 6026 adult COVID-19 patients admitted to two large centers in Saudi Arabia (Riyadh and Hafar Al-Batin cities) between April and June 2020 were retrospectively analysed.

Results: More than 23% of the study subjects with available data have died, enabling the prediction of mortality in our cohort. Markers that were significantly associated with mortality in this study were older age, increased D-dimer in the blood, higher counts of WBCs, higher percentage of neutrophil, and a higher chest X-ray (CXR) score. The CXR scores were also positively associated with age, D-dimer, WBC count, and percentage of neutrophil. This supports the utility of CXR scores in the absence of blood testing. Predicting mortality based on Ct values of RT-PCR was not successful, necessitating a more quantitative RT-PCR to determine virus quantity in samples. Our work has also identified age, D-dimer concentration, leukocyte parameters and CXR score to be prognostic markers of the OS of COVID-19 patients.

Conclusion: Overall, this retrospective study on hospitalised cohort of COVID-19 patients presents that age, haematological, and radiological data at the time of diagnosis are of value and could be used to guide better clinical management of COVID-19 patients.

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Introduction

A novel coronavirus has recently emerged into human populations, which was first identified in December 2019 in Wuhan, China and has spread globally causing a global pandemic [1–3]. This virus is named Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and cause the coronavirus disease 2019 (COVID-19) [1–5]. The virus has infected more than 173 million in the pandemic and caused over three million deaths in more than 200 countries worldwide [6–8].

The clinical manifestation of COVID-19 includes fever, non-productive cough, hypoxia, runny nose, sore throat, fatigue and dyspnea [9–11]. Other less common symptoms reported to be vomiting, diarrhoea, nausea, abdominal pain [9,12,13]. The virus transmission occurs through breathing droplets carrying the virus from cough or sneezing via close contact [14,15] especially from symptomatic patients [16], and contaminated items or surfaces can also be a source of COVID-19 infection [4,16].

Laboratory diagnosis is achieved mainly by reverse transcription-polymerase chain reaction (RT-PCR) tests to detect parts of the viral genes in respiratory samples [17]. Other samples such as stool or saliva could also be used but with less reliability and accuracy [18–20]. RT-PCR is designed to target parts of different viral genes; such as those encode for spike, nucleocapsid, and envelope proteins [17].

The prognosis of COVID-19 has been shown to vary significantly among patients. The majority of patients exhibit a mild form of the disease with no major complications and full recovery after receiving proper clinical care, whereas a small proportion of the patients show a severe disease manifestation with rapid progression to high-risk of death [4,21]. COVID-19 was found to be associated with abnormal readings in haematological, biochemical, inflammatory, and immunological tests [22]. Interestingly, some of these abnormalities were proposed to distinguish between mild and severe cases [22]. For example, lymphopenia was observed in 80% of COVID-19 patients with critical conditions [23], while only 20% of mild cases showed lymphopenia, suggesting low count of lymphocytes could serve as a poor prognostic marker [24,25]. In addition, percentages of basophils, eosinophils and monocytes, were reported to be lower in severe cases of COVID-19 compared to non-severe cases [26]. Inflammatory proteins such as C-reactive protein (CRP) and main constituents of plasma proteins like albumin were significantly associated with the severe form of the disease [21,22]. Taken together, these studies suggested that routine test performed in a standard diagnostic lab (e.g., haematology, serology and biochemistry) hold valuable information that may help to predict the prognosis of the disease.

Given the heterogeneous clinical outcomes of COVID-19, identification of biomarkers of the disease that can be measured at the time of infection (baseline) is useful and urgently needed to enable clinicians to predict the disease prognosis. Early knowledge of the disease prognosis is likely to direct early actions, leading to reduced complications and mortality rate. Here, clinical data of COVID-19 patients from Saudi Arabia were retrospectively analyzed in order to search for (i) biomarkers associated with the mortality of the disease, and (ii) predictors of the OS of the patients.

Methods

Data collection

Complete blood count, D-dimer, viral load, and vital status of hospitalized adult patients with confirmed COVID-19 by PCR testing at the time of diagnosis in King Abdulaziz Medical City, Riyadh and King Khalid General Hospital, Hafar Al-Batin city were ret-

respectively retrieved from the electronic health records for the periods of 02/April/2020 to 18/June/2020 (IRB approval number: RC/357/R) and 26/June/2020 to 16/September/2020 (IRB approval number: 1442-651036), respectively. Chest X-ray scans of those patients were collected from the hospital picture archiving and communication system and annotated based on the presentation of ground glass opacity/consolidation by four radiologists (experience 3–15 years). For viral load, the data were stored in a narrative text, so we created an in-house python script to extract S, N1, N2 and E genes Ct values using regular expression algorithm.

Calculation of chest X-ray score

Similar to Toussie et al. [27], we divided the chest X-ray (CXR) into 12 regions (6 for each lung) to score upper, middle, and lower lung zones. For each region the score ranges between 0–2, where 0 represents no manifestation of GOO/consolidation, and 2 represent severe opacity. The 12 lung zones severity values are then added to generate one score between 0–24 for each patient as a measure of lungs GOO/consolidation severity.

Statistic analysis

Excel software and coefficient of determination test (R^2) were used to determine the correlation degree between the Ct values of the diagnostic genes of COVID-19. Unpaired student *t*-test was applied using Prism Graph Pad software to calculate the statistical significance of biomarkers associated with mortality of COVID-19. One-Way ANOVA test with Prism Graph Pad software were employed to determine the statistical significance of biomarkers associated with CXR score. Kaplan–Meier curve and log-rank test were used for the OS analysis.

Results

Age and locations of the study's patients

In the present work, a retrospective study on adult hospitalized patients with COVID-19 between 2/April/2020 and 16/September/2020 in two regions of Saudi Arabia (SA); Riyadh City (central region of SA; National Guard Hospital) and Hafar Al-Batin city (North region of SA; King Khalid General Hospital) was conducted. The total number of patients was 6026 of whom 3226 (53%) were females and 2799 (47%) were males. The median age of the patients was 38 years with the range being from 15 years to 106 years.

Correlation analysis of COVID-19 diagnostic genes

One of the most common diagnostic tools of COVID-19 is the detection and relative quantification of SARS-CoV-2 genes; such as spike gene (gene S), envelop gene (gene E), nucleocapsid genes (N1 and N2 genes), using RT-PCR test. The data set used in this study contained viral loads of COVID-19 patients based on the following gene targets: gene E data for 5592 patients, gene S data for 5480 patients, gene N1 for 200 patients and gene N2 for 285 patients. Coefficient of determination test (R^2) was applied to examine the correlation between the Ct values of the target genes. The analysis showed a positive correlations of the Ct values of gene S, gene N1 and gene N2 with that of gene E. The highest correlation was observed for gene S with gene E ($R^2 = 0.94$, $n = 5119$; Fig. 1(A)). Gene N2 and gene E showed a lower degree of correlation ($R^2 = 0.83$, $n = 274$; Fig. 1(B)). The lowest correlation was between gene N1 and gene E ($R^2 = 0.50$, $n = 139$), which could be due to outlier samples that needs further investigations; Fig. 1(C). Given the high degree

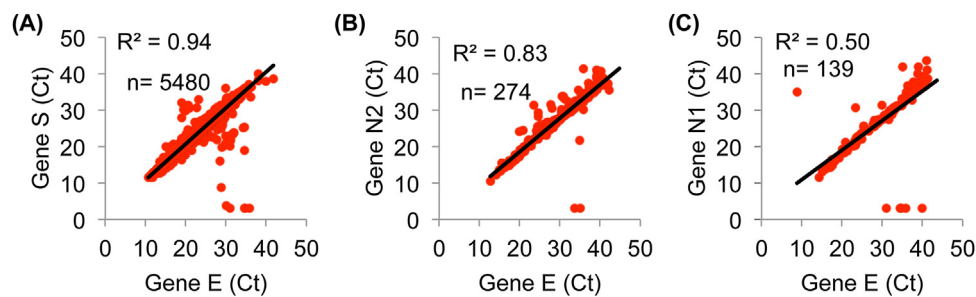


Fig. 1. Correlation analysis of the diagnostic target genes of COVID-19. The analysis was conducted using coefficient of determination test (R^2).

of correlation between gene S and E, it seems reliably practical to select one of these two genes for the diagnosis of COVID-19.

Biomarkers associated with mortality of COVID-19 patients

Of the 6026 patients, the follow up data (vital status; death or survival with full recovery) were available for 1683; with a severe disease in 398 patients resulting in death. On the other hand, 1285 patients exhibited mild symptoms followed by complete recovery. In order to search for biomarkers that identify COVID19 patients at high-risk of death, clinical data concerning patient age, viral load, haematological parameters, CXR score were studied in the deceased and survived patients. No significant differences between the two groups of patients was recorded for the red blood cells (RBCs) count, haemoglobin (Hb) concentration, mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC), haematocrit, platelets count or viral load, which was determined based on viral genes of S, E, N1 and N2. In contrast, age appeared to be an important determinant of the disease prognosis; the mean age was 61.4 years in the deceased patients compared to 53 years in the survived patients (Fig. 2(A), $p < 0.0001$). In addition, a significant association of D-dimer with the mortality was found; the D-dimer level was 3.3 $\mu\text{g/mL}$ in fatal cases versus 2 $\mu\text{g/mL}$ in survived cases (Fig. 2(B); $p = 0.003$). White blood cells (WBCs) count and the neutrophil percentage were associated with poor prognosis of the disease. The WBCs count was 9.3×10^9 cells/L with 73% neutrophils in the deceased patients versus 7.1×10^9 cells/L with 65.7% neutrophils in the survived patients (Fig. 2(C) and (E); $p < 0.0001$). On the other hand, increased percentage of lymphocytes, monocytes and eosinophils was characteristic of favourable prognosis of the disease. The proportions of lymphocytes, monocytes and eosinophils were 25%, 8.6% and 1% in survivors compared to 19%, 7% and 0.8% in dead patients (Fig. 1(D), $p < 0.0001$; Fig. 1(F), $p < 0.0001$; Fig. 1(G), $p = 0.03$). CXR score appeared to associate with mortality of COVID-19; the CXR score was 5.4 in the fatal cases as opposed to 2.8 in the survived cases (Fig. 1(H), $p < 0.0001$). Taken together, these findings revealed informative roles of patient age, CBC data and CXR score in the prognosis of COVID-19.

Biomarkers associated with CXR score in COVID-19 patients

CXR score has been proposed to serve as a monitoring tool for the clinical course of COVID-19. Given the significant association of CXR score with the mortality of COVID-19 shown in Fig. 1(H) and by others [28,29], association between CXR score and patient age, viral load or blood parameter was investigated. Patients were divided into six groups on the basis of CXR score (0, 1–3, 4–6, 7–9, 10–12 and 13–22). The CXR score = 0 indicated no presentation of ground glass opacity/air space consolidation, while CXR = 22 denotes severe presentation of ground glass opacity/air space. The CXR scores were available for 2018 patients. The analysis showed no significant association of RBCs count, Hb concentra-

tion, MCV, MCH, MCHC, haematocrit and viral load (S, E, N1 and N2 genes) with the CXR score. However, older age (Fig. 3(A); $p < 0.0001$) and increased values of D-dimer concentration (Fig. 3(B); $p = 0.01$), count of WBCs (Fig. 3(C); $p < 0.0001$), neutrophil percentage (Fig. 3(E); $p < 0.0001$), count of platelets (Fig. 3(L); $p = 0.0006$), appeared to be risk factors associated with higher CXR score. In contrast, high percentages of lymphocytes (Fig. 3(D); $p < 0.0001$), monocytes (Fig. 3(F); $p < 0.0001$), eosinophils (Fig. 3(G); $p < 0.0001$), and basophils (Fig. 3(H); $p < 0.0001$) were associated with lower CXR scores.

Prediction of the overall survival in COVID-19 patients

The potential of the above clinical data to predict the OS of COVID-19 patients was evaluated. The OS data were available for 1084 patients. Initially, the median value of the clinical data was used to divide patients into two groups (high group (HG) > median value; and low group (LG) < median value). Next, the OS data of the two groups were compared for each clinical parameter. RBCs count, Hb concentration, MCV, MCH, MCHC, haematocrit and viral load (genes S, E, N1 and N2) did not significantly predict the OS of COVID-19 patients. Nevertheless, age, D-dimer, leukocyte parameters and CXR score were significantly informative of the patients' OS (Table 1). Next, an effort was made to improve the ability of the previous parameters to predict the OS of COVID-19 patients by using the "Cutoff Finder", which is an online tool that searches for the optimal cutoff value to generate the most significant p value and hazard ratio (HR) using log-rank test [30]. The cut-off values produced by "Cutoff Finder" significantly enhanced the capability of the parameters to predict the OS (Fig. 4). For example, on the basis of the median value of patients' age, the HG (age > median value of all patients' age) possessed a median OS of 21 days compared to 19 days for the LG (age < median value of all patients' age). The calculated HR of the HG versus LG was 1.30 ($p = 0.01$; Table 1). In contrast, the cut-off value of age determined by "Cutoff Finder" separated the patients into HG (median OS = 12 days) and LG (median OS = 22 days) with HR of HG versus LG = 3.4 and $p < 0.0001$ (Fig. 4(A)). Among the markers, the best predictors of short OS appeared to be increased age (Fig. 4(A)) and high neutrophil % (HR of HG versus LG = 3.4; $p < 0.0001$; Fig. 4(D)). To the contrary, elevated monocyte % (HR of HG versus LG = 0.3; $p < 0.0001$; Fig. 4(E)) and raised lymphocyte % (HR of HG versus LG = 0.45; $p < 0.0001$; Fig. 4(C)) were found to be the best markers for long OS.

Discussion

In this retrospective study, data from adult COVID-19 hospitalised patients were analysed in order to search for biomarkers that identify patients at high-risk of death. The evaluated parameters included age, RT-PCR-based viral load, D-dimer, CBC, and CXR score. The cohort involved data on RT-PCR tests that were performed on various viral genetic targets; however, the most used

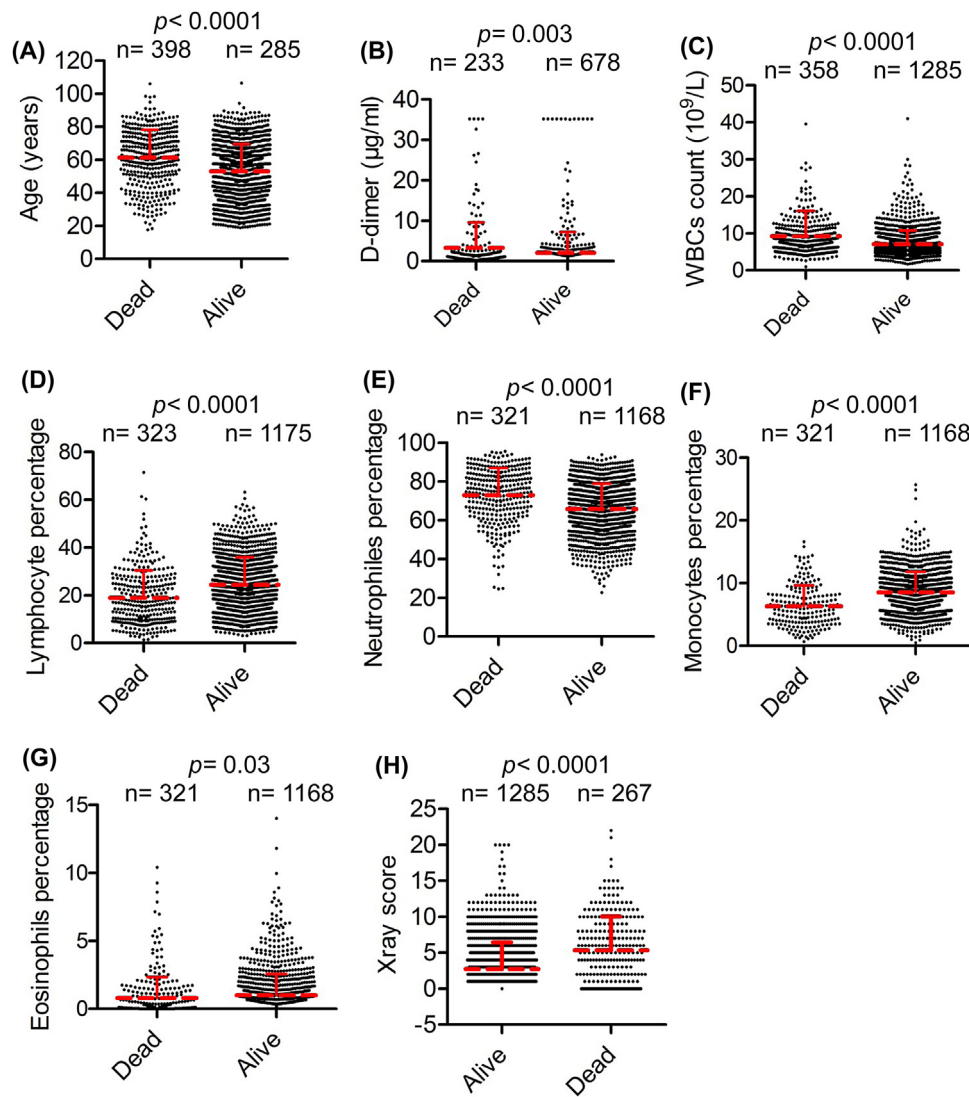


Fig. 2. Biomarkers associated with mortality in COVID-19 patients. Clinical data concerning age (A), d-dimer concentration (B) WBCs parameters (C–G) and chest X-ray (CXR) score (H) were compared in the deceased patient (Dead) and survivors (Alive).

Table 1
Median value-based prognostic markers for the prediction of overall survival in COVID-19 patients.

Marker	Median OS	Hazard ration (high group versus low group)	p Value	Number of patients
Age	HG = 21 day LG = 19 days	1.30	0.01	1048
D-dimer	HG = 21 day LG = 30 days	1.4	0.02	961
WBCs count	HG = 19 day LG = 24 days	1.4	0.002	1044
Lymphocyte percentage	HG = 27 day LG = 18 days	0.6	<0.0001	975
Neutrophil percentage	HG = 17 day LG = 30 days	1.75	<0.0001	971
Monocyte percentage	HG = 31 day LG = 18 days	0.67	0.0007	971
Eosinophil percentage	HG = 25 day LG = 19 days	0.7	0.003	971
CXR score	HG = 24 days LG = 33 days	1.5	0.002	953

Median value of the markers was used to divide the patients into two groups; high group (HG) > median value and low group (LG) < median value. Next, Kaplan–Meier curve with long-rank test was used to compare the OS in the two groups. OS: overall survival; CXR: chest X-ray.

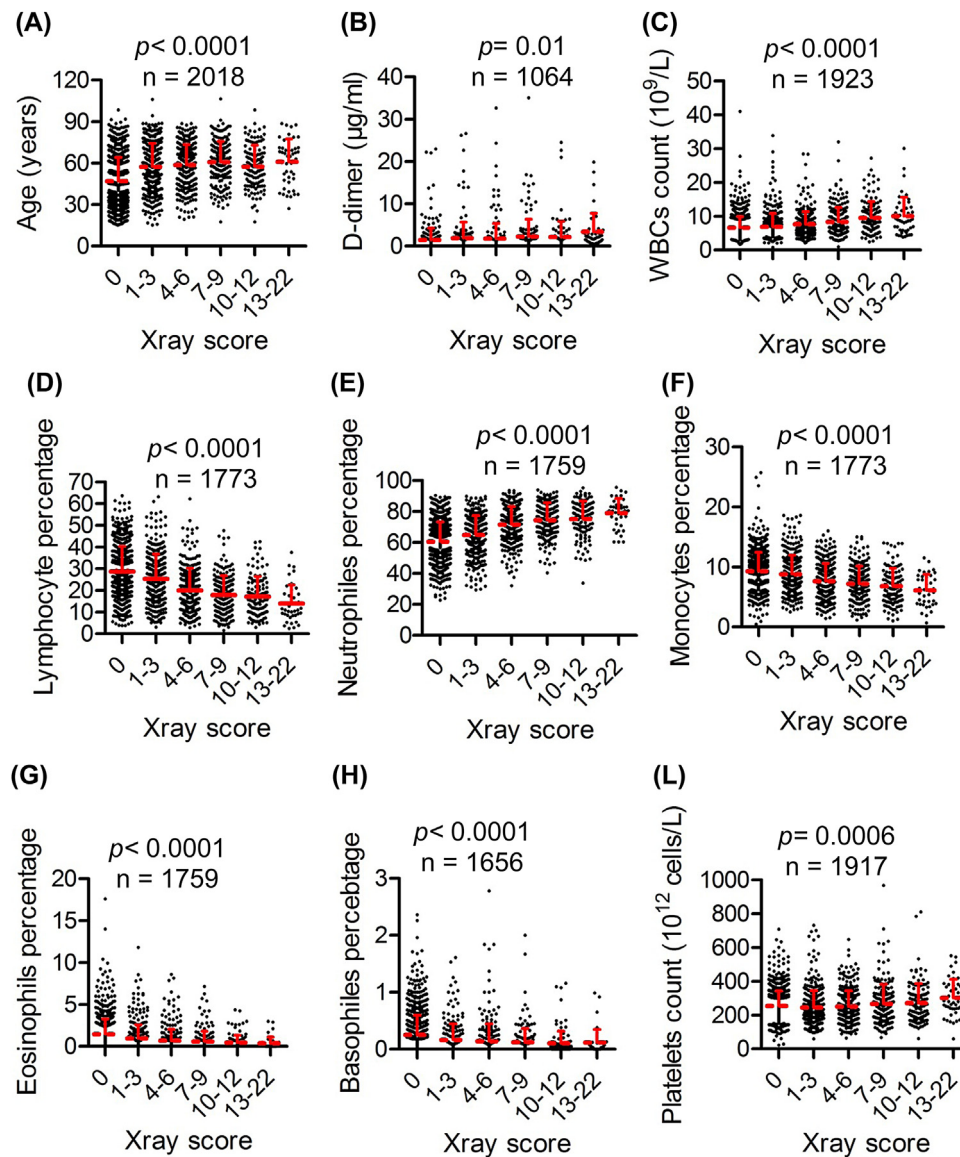


Fig. 3. Biomarkers associated with CXR score in COVID-19 patients. On the basis of the severity of CXR score, patients were divided into six groups and the clinical data of age (A), D-dimer concentration (B), leukocyte parameters (C–G) and platelet count (L) were studied.

targets were S and E. Ct values of RT-PCR that targets the E gene correlated with each of the other targets, such as S, N1, and N2. The strongest correlation was observed between Ct values of S and E genes. Using the spike gene as confirmatory is widely applicable and supported by many studies as it can differentiate between wild-type SARS-CoV-2 and variants of concerns that mutate and spread in various geographical areas such as variants B.1.1.7 from the U.K., B.1.351 from South Africa, and P.1 lineage from Brazil [31–34]. While the CDC suggests testing N1, N2, and RdRp as primary targets, the WHO support using the E as primary target and RdRp as confirmatory [35,36]; however, more targets and assays are widely used in various countries by different diagnostic providers. Our analysis supports the utility of the E and S targets in a sequential testing method where E serve as the primary target and S as the confirmatory target.

More than 23% of the study subjects with available data have died, but this does not reflect the mortality rate as data were not available for large number of the subjects. In addition, this study focused on hospitalised patients who have mainly moderate to severe conditions, this is similar to other studies that focused

on hospitalised cases where mortality rate is high [37]. Markers that were associated with mortality in our study were older age, increased D-dimer in the blood, higher counts of WBCs, higher percentage of neutrophil, and a higher CXR score. The CXR scores were also positively associated with age, D-dimer, WBC count, and percentage of neutrophil. This supports the utility of CXR scores in the absence of blood testing. More than thirty studies have previously reported the potential of neutrophil and D-dimer as well as other markers such as CRP, platelet, and neutrophil/lymphocyte ratio as strong predictors of COVID-19 prognosis to severe conditions and fatal outcomes [38]. Neutrophil counts in an earlier study [26] were observed to be as 4.3×10^9 cells/L in those who progressed to severe cases whereas in our cohort neutrophil was 73% of the total count of 9.3×10^9 cells/L in those who passed away, meaning neutrophil counts was on average around 6.7×10^9 cells/L. The higher count of neutrophil in our subjects could be because our cohort was based on hospitalised patients with mainly moderate to severe manifestation of the disease.

Our data did not show significant differences between deceased and survivors in RBCs count, Hb concentration, MCV, MCH, MCHC,

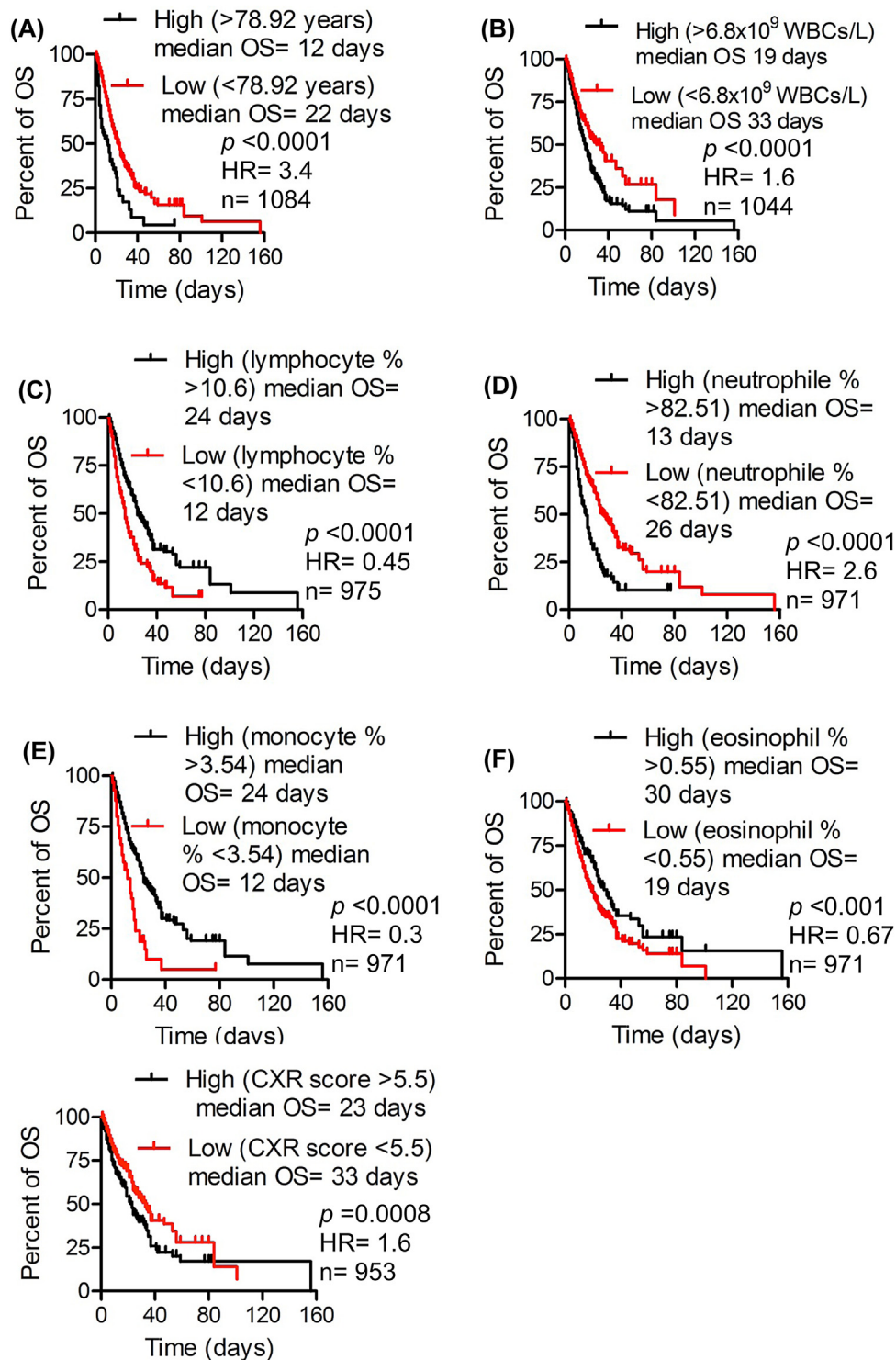


Fig. 4. Prognostic markers of OS in COVID-19 patients. Kaplan–Meier curve and log-rank test show that age (A), leukocyte parameters (B–F) and CXR score significantly predict the OS of COVID-19 patients. The cut-off value that was used to segregate patients into high and low groups was generated by “Cutoff Finder” for each clinical parameter.

or platelets count. Although platelet counts and RBC distribution width (RDW) were previously suggested to be associated with COVID-19 mortality, these reports have monitored the increase of biomarkers over the course of the infection and not only at the time of diagnosis [39,40].

The utility of viral load in predicting mortality has been reported previously [41]; however, our study is based on Ct values, which do not directly implies the magnitude of viruses in the samples;

and a further quantitative RT-PCR would be required to evaluate the precise viral load and its association with COVID-19 outcomes. There have been contradicting reports on the utility of Ct values in predicting the outcome of COVID-19 [42–45]. One report showed that higher viral RNA load in plasmas could predict mortality [46], but plasma viremia data were not available for our cohort.

Previous studies successfully predicted the OS based on clinical signs, co-morbidities, ICU length of stay, and socioeconomic con-

ditions [47–50]. Adding to this, our work identified age, D-dimer concentration, leukocyte parameters and CXR score to be prognostic markers of OS in COVID-19 patients. For example, older age appeared in our study to be the most significant predictor of short OS, whereas increased percentage of monocyte was the most significant indicator of long OS.

Ideally, a biomarker serves well when it can be assessed at time of diagnosis (baseline) to predict future clinical course of a disease. Interestingly, the data (D-dimer, CBC and viral load) studied here were generated at the time of diagnosis. Therefore, the biomarkers of mortality and OS, proposed here, could be of benefit for COVID-19 patients as they provide an early prediction of prognosis, which allows clinical decisions to be taken on time and provides better clinical management.

Conclusion

In the present work, clinical data of hospitalised COVID-19 adult patients from two centres in different regions of SA were retrospectively collected and studied with view of identifying biomarkers of mortality and predictors of OS. WBCs-related parameters, such as count of WBCs and neutrophil percentage, D-dimer and CXR score were found to significantly associate with the mortality of COVID-19 patients. These findings do not only propose biomarkers of mortality, but also provide insight into the pathological variation between patients with mild form of the disease and those with severe clinical outcomes. The integration of CBC data with CXR score showed a significant association of haematological parameters with increased CXR score, supporting the use of CXR score in the absence of blood testing. The present work identified patient's age, haematological parameters and CXR score to be useful prognostic markers that predict the OS of patients. Overall, our study adds to the knowledge of utility of laboratory findings in the context of COVID-19 prognosis; and specifically support the usefulness patient's age, haematological data, and CXR score for the better guide of clinical management of COVID-19 patients.

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Competing interests

None declared.

Ethical approval

The study was approved by the IRB at KAIMRC for the study RC/357/R and the IRB at the health ministry for the study 1442-651036.

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