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ORIGINAL ARTICLE

Combination of cycle threshold time, absolute lymphocyte count and neutrophil:lymphocyte ratio is predictive of hypoxia in patients with SARS-CoV-2 infection

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Background: There is currently no clinically validated biomarker to predict respiratory compromise in sudden acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Cycle threshold time (Ct), absolute lymphocyte count (AL) and neutrophil:lymphocyte ratio (NLR) have been previously evaluated for this purpose. We hypothesized that the combination of these parameters at presentation may be predictive of hypoxia (oxygen saturation <92%).

Methods: Data were collected on 118 patients with SARS-CoV-2 infection between May 2020 and April 2021. Demographics, clinical parameters and laboratory and radiological investigation results were recorded. Respiratory compromise (RC) was defined based on symptoms and signs, hypoxia and chest X-ray abnormalities.

Results: RC occurred in 61 (51.7%) of patients. The Ct, AL and NLR at median day 3 of illness were significantly different between patients with and without RC (Ct, RC vs not: 19.46±2.64 vs 22.62±3.37, p=0.0001; AL, RC vs not: 531.49±289.09 vs 764.69±481.79, p=0.0001; NLR, RC vs not: 3.42±0.75 vs 2.59±0.55, p=0.0001). Receiver operating characteristics analysis showed that a Ct <19.9, AL $<630.8\times10^3/\mu$L and NLR >3.12 at median day 3 of symptoms was predictive of hypoxia on day 7 of illness (area under the curve 0.805, sensitivity 96.7%, specificity 69.1%). The predictive value for the parameters combined was significantly superior to their individual predictive power.

Conclusions: Ct, AL and NLR used in combination on day 3 of symptoms are predictive of hypoxia on day 7 of SARS-CoV-2 illness.

Keywords: absolute lymphocyte count, cycle threshold time, neutrophil:lymphocyte ratio, respiratory compromise, SARS-CoV-2

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that causes coronavirus disease 2019 (COVID-19) and was first identified in Wuhan, China in December 2019.[1] While COVID-19 can have a variety of presentations, upper respiratory tract symptoms are the most common.[2–4] Hypoxia secondary to COVID-19 infection is a result of an acute inflammatory response affecting the lungs.[5,6] The incidence of COVID-19-related hypoxia ranges from 20 to 40% and is the main cause of mortality related to COVID 19.[6–10] Early identification of COVID-19-related hypoxia is therefore essential.

Respiratory compromise (RC) in COVID-19 is defined in Sri Lanka based on national guidelines.[11] Specific parameters include epidemiological history, clinical symptoms, vital signs (respiratory rate 20–30 breathes/min, heart rate 100–120 bpm, oxygen saturation on room air <92% by pulse oximeter) and chest X-ray (CXR)

findings. Relevant CXR findings included ground glass opacities, peripheral consolidation and effusions.[11]

Granulocyte colony stimulating factor (G-CSF), interferon-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1) and inflammatory macrophage protein-1α have been proposed as predictors of severe COVID-19.[12] However, these parameters have not been validated for clinical use and are not readily available in developing countries. More easily accessible investigations that are proposed to predict the severity of COVID-19 include troponin, creatine kinase MB, N-terminal prohormone of brain natriuretic peptide, C-reactive protein (CRP) and lactate dehydrogenase.[13,14] The predictive value of these biomarkers was demonstrated during the first week of hospital admission.[15] The standard method for diagnosis of COVID-19 is via real-time reverse transcription polymerase chain reaction (RT-PCR).[16] Real-time RT-PCR cycle threshold (Ct) values represent the number of amplification cycles required for the target gene to exceed a threshold level. Ct values are therefore inversely related to viral load[17] and lower Ct values are associated with adverse outcomes in COVID-19.[18]

Increased lymphocyte apoptosis and T cell deregulation (attributed to high levels of interleukin-6 [IL-6]) as well as Fas–Fas ligand interactions may contribute to the immune deregulation leading to COVID-19-related RC.[19,20] Reduced $CD4^+$ and $CD8^+$ T cell subsets were also shown to be predictors for severe COVID-19.[10] A low absolute lymphocyte (AL) count on admission was associated with poor outcomes in a meta-analysis of patients with COVID-19 and was predictive for which patients may benefit from steroid therapy.[21,22] Neutrophil activation combined with older age and an increase of neutrophil extracellular traps contribute to COVID-19-related RC.[23–25] Also, neutrophilia as well as an elevated neutrophil:lymphocyte ratio (NLR) have been reported as predictive factors for adverse outcomes in COVID-19.[25–28] We hypothesized that Ct, AL and NLR, when used collectively, may have a stronger predictive value for respiratory compromise than when these parameters are used individually.

Methods

Study design

A retrospective study was conducted on adult patients admitted to Nawaloka Hospital, Colombo, Sri Lanka with confirmed SARS-CoV-2 infection between May 2020 and April 2021.

Study population

We included 118 patients (>18 y of age) with SARS-CoV-2 infection confirmed by RT-PCR from nasopharyngeal swabs (AccuPower SARS-CoV-2 RT-PCR kit, Bioneer, Daejeon, South Korea).[29] Positive test results were re-analysed with the RealStar SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany)[30] for confirmation. The reliability of the results was ensured through internal quality control measures as well as participation in external quality assurance schemes in collaboration with the Medical Research Institute of Sri Lanka. The onset of illness was determined based on guidelines from the Ministry of Health of Sri Lanka. Relevant symptoms included fever, cough, difficulty breathing and sore throat.[31]

Patients <18 y of age and those with immunosuppression were excluded from the study. Immunosuppression was defined as the presence of human immunodeficiency virus infection, solid organ or stem cell transplantation, neutropenia or ongoing immunosuppressive treatment.

Data collection

Demographic, clinical and laboratory data

Demographic and clinical parameters, including presenting symptoms, vital signs and laboratory and radiological investigations, were extracted by a trained study team member from the available medical records. Pulse oximetry was performed based on the manufacturer's guidelines (see Supplementary Data 1). CXR images were analysed by two experienced consultant radiologists who were blinded to the clinical data and final decisions were reached by consensus. For disagreement in interpretation between the two radiologists, a third radiologist adjudicated a final decision. AL and neutrophil count data were extracted from the XN-1000 automated full blood count analyser (Sysmex, Kobe, Japan).

Data analysis

Continuous variables were described using mean and standard deviation (SD) values. Differences between groups were compared using Student's t-test (parametric data) or the Mann–Whitney U-test (non-parametric data). Categorical data were expressed in total numbers and percentages and compared using a Z-test for two proportions and were compared using the χ^2 test. For receiver operating characteristics (ROC) curve analysis, specific cut-off values for AL, Ct and NLR were used to determine the area under the curve (AUC) and sensitivity and specificity values. p-Values <0.05 were considered statistically significant. The average oxygen saturation level on day 7 of illness was used as the independent variable for the ROC analysis. Data were analysed using the SPSS version 16 (SPSS, Chicago, IL, USA) and Stata version 12 (StataCorp, College Station, TX, USA).

Results

We extracted records of 118 patients, 53.4% males, with a mean age of 50.22±15.11 y. Half of the patients 59/118 (50.0%) did not have any comorbidities and were admitted on day 3 of illness. The median length of hospital stay was 14 d. The most common presenting symptom was fever (84/118 [71.2%]) and intensive care unit (ICU) admission was required in 61/118 (51.7%) patients.

Patients were treated based on national guidelines[11] and World Health Organization guidelines.[31] Patients with RC comprised 51.7% of cases (61/118), all of whom were treated with oxygen, intravenous dexamethasone and prophylactic low molecular weight heparin. Among the patients with RC, 41/61 (67.2%) required ICU care and 50/61 (81.9%) had mean oxygen saturation levels of 85% on day 9 of the illness. Treatment for these patients comprised high-flow oxygen for 31/61 (50.8%), continuous positive airway pressure (CPAP) for 14/61 (22.9%) and invasive ventilation for 5/61 (8.2%).

Table 1. Demographic characteristics of the study population

Variable	n (%)
Age (years)	
< 30	15 (12.7)
31–40	20 (16.9)
41–50	21 (17.8)
51–60	25 (21.2)
61–70	29 (24.6)
≥71	8 (6.8)
Gender	
Male	63 (53.4)
Female	55 (46.6)
Comorbidities	
Hypertension	37 (31.3)
Diabetes	30 (25.4)
Dyslipidaemia	12 (10.2)
Ischaemic heart disease	9 (7.6)
Chronic kidney disease	8 (6.7)
Chronic liver disease	8 (6.7)
Asthma	6 (5.1)
COPD	5 (4.2)
None	59 (50.0)
Days following onset of symptoms	
3	96 (81.3)
4	18 (15.2)
5	4 (3.4)
Length of hospital stay	
<14	75 (63.5)
14–16	37 (31.4)
≥17	6 (5.1)
Symptoms	
Fever	84 (71.2)
Headache	54 (45.8)
Cough	93 (78.8)
Dyspnoea	61 (51.7)
Arthralgia/myalgia	84 (71.2)
Diarrhoea	28 (23.7)
Sore throat	78 (66.1)
Setting of care	
Non-respiratory compromised (n=57 [48.3%])	
General ward	57 (100)
ICU	0 (0)
Respiratory compromised (n=61 [51.7%])	
General ward	20 (32.8)
ICU	41 (67.2)
Mode of oxygen therapy for respiratory-compromised patients (n=61)	
Face mask	11 (18.1)
High flow	31 (50.8)
CPAP	14 (22.9)
Invasive ventilation	5 (8.2)
Medication management for respiratory-compromised patients (n=61)	
Intravenous dexamethasone 6 mg daily for a median of 10 d	61 (100)
Two doses of intravenous tocilizumab (median dose of 400 mg daily)	46 (75.4)
Intravenous antibiotic	61 (100)
Subcutaneous low molecular heparin (median dose of 40 mg daily)	61 (100)
Oral azithromycin 500 mg daily	61 (100)
Outcome	
Non respiratory compromised (n=57)	
Recovered	5 7(100)
Death	0 (0)
Respiratory compromised (n=61)	
Recovered	51 (83.4)
Death	10 (16.4)

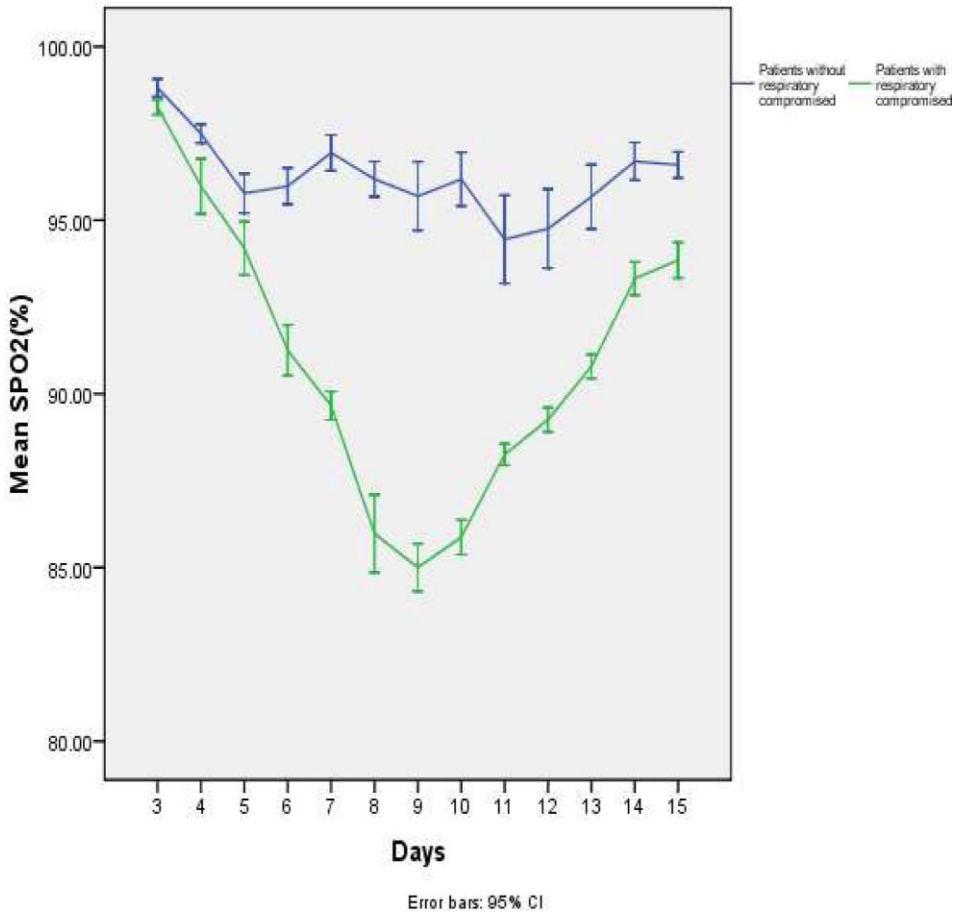


Figure 1. Variation of oxygen levels by pulse oximetry (on room air) of patients with and without respiratory compromise.

Intravenous antibiotics were given to patients with elevated procalcitonin, positive sputum or blood culture and/or elevated CRP in whom concurrent bacterial infection was suspected. Tocilizumab was administered to patients who had the following parameters: ferritin ≥600 ng/mL, CRP ≥80 mg/L[32] and IL-6 >37.65 pg/ml.[33] We recorded 10 (16.4%) deaths during the study period, all of which occurred among the RC group. Two or more comorbidities were reported in 39/61 (63.9%) patients with RC, in contrast to the non-respiratory-compromised group, where 51/57 (89.5%) had only one or no comorbidities. The demographic and clinical characteristics of the study cohort as well as details of treatment are summarized in Table 1.

Respiratory rate on admission (19.87±2.87 vs 17.12±1.41; p=0.0001) and length of hospital stay (14.21±1.53 vs 10.51±1.85; p<0.0001) were significantly higher in the RC group. There was a statistically significant difference in oxygen saturation levels between patients with and without RC from day 3 onwards. Figure 1 shows the variation of oxygen saturation by pulse oximeter in patients with and without RC. Table 2 shows the demographic characteristics, admission vital signs and vaccination status of patients with and without RC. All patients in the RC group had CXR abnormalities, including ground glass opacities, consolidation or effusions. Table 3 summarizes the laboratory and radiological findings of patients with and without RC.

While the AL on admission was significantly lower (531.49±289.09×$10^3/\mu$L vs 764.69±481.79×$10^3/\mu$L; p=0.0001), the NLR was significantly higher (3.42±0.75 vs 2.59±0.55; p=0.0001) in the group with RC. The Ct value was also significantly lower in the RC group (19.46±2.64 vs 22.62±3.37; p=0.0001). ROC analysis demonstrated that a Ct value <19.9, AL <630.8×$10^3/\mu$L and NLR >3.12 were predictive of hypoxia (oxygen saturation on air by pulse oximeter <92%) on day 7 of illness. The Ct, AL and NLR thresholds when used as a combined modal showed a stronger predictive power for hypoxia on day 7 of illness than when used individually (AUC 0.805, sensitivity 96.7%, specificity 69.1%). These data are summarized in Figure 2. The Ministry of Health guidelines in Sri Lanka define critical or severe COVID-19 based on oxygen saturation <92%. In our cohort, the mean oxygen saturation was 92% before day 7 and 90% on or after day 7. We therefore chose day 7 as the time point at which we sought to predict hypoxia.

Discussion

We demonstrate for the first time that the combination of NLR, AL and Ct on day 3 of COVID-19 illness is predictive of hypoxia on

Table 2. Comparison of demographic characteristics, vital signs on admission and vaccination status of patients with and without respiratory compromise

Variable	Patients with respiratory compromise (n=61)	Patients without respiratory compromise (n=57)	p-Value
Age (years), mean±SD	51.30±14.48	49.07±15.18	0.427
Gender, n (%)			
Male	36 (59.1)	27 (48.9)	0.686
Female	25 (40.9)	30 (53.1)	
Comorbidities, n (%)			
0	7 (11.5)	37 (64.9)	**<0.0001**
1	15 (24.6)	14 (24.6)	
2	23 (37.7)	3 (5.3)	
3	16 (26.2)	3 (5.3)	
Length of hospital stay (days), mean±SD	14.21±1.53	10.51±1.85	**<0.0001**
Systolic blood pressure median day 3 of illness (mmHg), mean±SD	127.51±10.64	132.12±13.32	0.065
Diastolic blood pressure median day 3 of illness (mmHg), mean±SD	75.98±5.98	78.69±5.95	**0.028**
Heart rate/min median day 3 of illness, mean±SD	85.85±9.83	86.43±8.64	0.76
Respiratory rate/min median day 3 of illness, mean±SD	19.87±2.87	17.12±1.41	**<0.0001**
Vaccination status[a]			
None	50 (81.9)	21 (36.8)	**<0.0001**
1 dose	10 (16.4)	36 (63.2)	
2 doses	1 (1.7)	0 (0)	

[a]Oxford-AstraZeneca in all patients.
Significant values in bold.

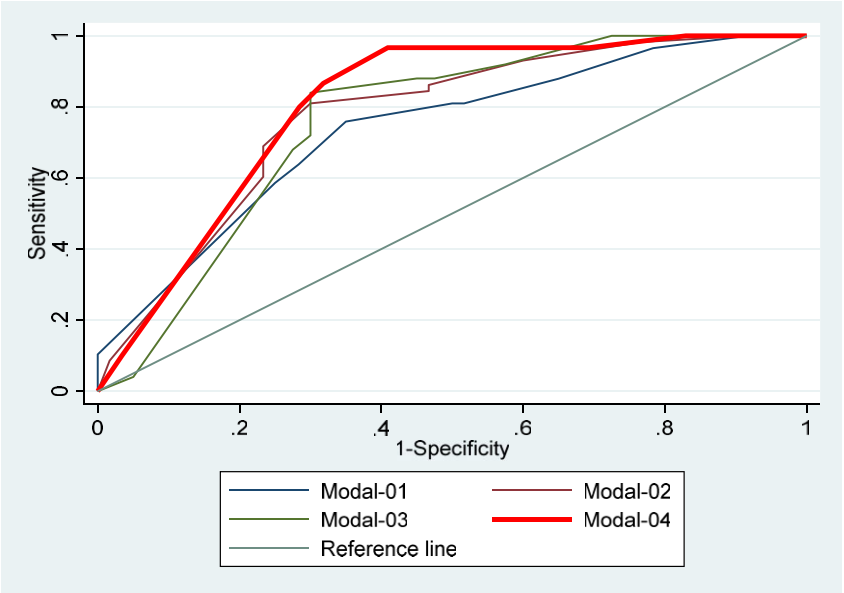
Table 3. Comparison of laboratory investigations, radiological findings and Ct among patients with and without respiratory compromise (median day 3 of illness)

Variable	Patients with respiratory compromise (n=61)	Patients without respiratory compromise (n=57)	p-Value
White cell count (10^9/L), mean±SD	2.73±1.23	3.36±1.78	**0.048**
Platelet count (10^9/L), mean±SD	142.2±35.37	145.63±35.62	0.640
Lymphocyte count (%), mean±SD	18.64±3.42	28.78±10.96	**<0.0001**
Absolute lymphocyte count ($10^3/\mu$L), mean±SD	531.49±289.09	764.69±481.79	**0.001**
Absolute neutrophil count ($10^3/\mu$L), mean±SD	1818.61±185.34	1989.34±278.32	**<0.0001**
Neutrophils (%)	66.5	58.3	0.359
NLR, mean±SD	3.42±0.75	2.59±0.55	**0.001**
Atypical lymphocyte count (10^9/L), mean±SD	0.18±0.11	0.24±0.12	0.328
C-reactive protein (mg/L), mean±SD	31.98±22.84	30.29±19.49	0.944
ALT (units/L), mean±SD	95.06±101.44	92.76±106.05	0.910
AST (units/L), mean±SD	142.62±149.85	153.47±192.89	0.875
PCV (%), mean±SD	38.80±4.94	40.08±4.46	0.186
Blood group, n (%)			
A	13 (21.3)	17 (29.8)	0.737
B	16 (26.2)	14 (24.6)	
O	26 (42.6)	22 (38.6)	
AB	6 (9.8)	4 (7.0)	
CXR findings, n (%)			
Ground glass opacities	61 (100.0)	0 (0)	N/A
Peripheral consolidations	21 (34.42)	1 (1.75)	**<0.0001**
Effusion	16 (26.22)	2 (3.5)	**<0.0001**
Ct, mean±SD	19.46±2.64	22.62±3.37	**<0.0001**

ALT: alanine transaminase, AST: aspartate aminotransferase, PCV: packed cell volume.
Significant values in bold.

day 7 of disease. While Ct values <20 at diagnosis were proposed as a marker of severity of COVID-19, this has not been consistently demonstrated across studies.[34,35] However, the Ct values proposed by other groups as predictive of severe disease are similar to those we identified.[36,37] Importantly, none of these studies looked at the combined predictive power of the Ct value and haematologic parameters.

Lymphopenia has been established as a feature of severe COVID-19 and a predictor of disease severity.[25,38,39] The predictive AL cut-off varies between studies, with values ranging



Modal 01: Ct value of less than 19.9, Modal 02: lymphocyte count less than 630.8 x $10^{\wedge 3}$/ μL, Modal 03: NLR more than 3.12 and Modal 04: Combination of Modal 01, 02 and 03

Predictor	AUC	95%CI of AUC	Sensitivity	Specificity	PPV	NPV	Youden's index
Modal 01: Ct value of less than 19.9	0.734	0.645-0.824	75.9	65.0	74.5	70.0	0.439
Modal 02: lymphocyte count less than 630.8μL	0.774	0.689-0.860	81.0	70.0	72.5	65.9	0.281
Modal 03: NLR more than 3.12	0.760	0.643-0.876	84.0	70.0	81.3	77.5	0.596
Modal 04: Combination of Modal 01,02 and 03	0.805	0.725-885	96.7	69.1	88.9	74.2	0.341

AUC: Area under the Curve, PPV: Positive predictive value, NPV: Negative predictive value.

Figure 2. The Ct value, AL count and NLR on day 3 of illness as predictors of oxygen saturation <92% on day 7 of illness.

from $<950\times10^3/\mu$L to $<600\times10^3/\mu$L.[40–44] These findings were confirmed in a systemic review and meta-analysis showing that COVID-19 patients with a good outcome had a significantly higher AL on admission than those with a poor outcome (mean difference between groups was $361.06\times10^3/\mu$L).[22] The AL values proposed by these studies are similar to those we report in this study. While a high lymphocyte:CRP ratio has also been proposed as an adverse prognostic factor in COVID-19, the non-specific nature of CRP may affect the routine applicability of this parameter.[45]

High NLR values have been reported in severe COVID-19, with thresholds of 5,[28] 3.3 and 4.7 being proposed as predictors of severity.[40] These values are similar to what we have proposed as predictive in our cohort and were also shown to be predictive at day 3 of symptoms. The similarity of predictive AL and NLR values between published data and ours suggests that the haematologic parameters in South Asian COVID-19 patients are similar to those of their East Asian counterparts.[25]

The mean age of patients with RC in our study (51.30±14.48 y) was similar to that reported by other groups.[41] We demonstrated that the patients with more than two comorbidities were at higher risk of RC, which is in keeping with results from other countries.[41–43] In contrast to other reports from Asia, we found no gender disparity in COVID-19 severity within our cohort.[46] In keeping with published data, we demonstrated that vaccinated individuals appear to have less severe COVID-19 disease, however, the relatively small numbers preclude a definitive comparison.[47,48] There is a paucity of data on the timing of hypoxia in COVID-19

patients. We found that severe hypoxia, defined by oxygen saturation <92%, occurred most commonly on day 7 of illness. These findings need to be validated in independent cohorts.

Limitations of our study include the fact that it was a retrospective analysis conducted at a single centre in Sri Lanka. Our findings need to be validated prospectively in larger independent cohorts within and outside South Asia. Future studies are required evaluating these parameters in conjunction with Ct, AL and NLR values as predictive tools for severe COVID-19. With expanding vaccination programs across the globe, how these predictive thresholds apply to fully vaccinated cohorts of patients would also be an important area to study.

Conclusions

We propose that a Ct value <19.9, AL $<630.8\times10^3/\mu$L and NLR >3.12 on day 3 of symptoms is predictive for hypoxia on day 7 of COVID-19 illness. These are readily available parameters that can be used routinely in developing countries in a pandemic setting. The results of our study are of clinical relevance to clinicians managing COVID-19 in resource-limited settings, where this combinations of parameters may provide a low-cost means of triaging patients.

Supplementary data

Supplementary data are available at *Transactions* online.

Authors' contributions: VA, SDM, SLS, APS, RM, TS, AF, CDM and LG conceptualized the study. VA, RSW, SM and PDM collected the data. VA, SDM, SLS and RM analysed the data and wrote the manuscript. VA, LC, CDM, SLS, AF and SDM conducted critical review and editing of manuscript. All authors read and approved the final manuscript. VA and SDM are guarantors of the paper.

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Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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