Original Article





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Clinical, laboratory and high-resolution computed tomography (HRCT) thorax profile of reverse transcription polymerase chain reaction (RT-PCR) negative COVID-19 suspects with moderate to severe disease

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Abstract:

BACKGROUND: Diagnostic dilemma arises when patients with clinical suspicion of COVID-19 disease having moderate-to-severe respiratory symptoms yield negative result for COVID-19 in reverse transcription polymerase chain reaction (RT-PCR). This study evaluated the clinical, laboratory and HRCT thorax findings among RT-PCR-negative COVID-19 suspects with moderate-to-severe disease.

MATERIALS AND METHODS: A hospital-based retrospective observational study was conducted between July 2021 to December 2021, among 60 moderate and severe symptomatic COVID-19 suspects admitted in the severe acute respiratory illness (SARI) ward and intensive care unit (ICU), who were negative for COVID-19 in RT-PCR. Data were abstracted from the medical records section of the hospital using a predesigned data abstraction form and presented by descriptive statistics.

RESULTS: Mean age of study participants was 55.5 years (SD 14.1 years), and majority were males (n = 43, 71.7%). Common presenting symptoms were fever (n = 60, 100%), dyspnea (n = 57, 95%), and cough (n = 54, 90%). The common laboratory findings were rise of C-reactive protein (n = 60, 100%), NLR (n = 49, 81.7%), d-dimer (n = 47, 78.3%), ferritin (n = 46, 76.7%), and LDH (n = 40, 66.7%). HRCT scan of thorax revealed ground glass opacities with or without consolidations located bilaterally with diffuse or peripheral distribution, interlobar septal thickening (n = 43, 74.1%), vascular thickening (n = 35, ≥ 58.3 %), and sub-pleural lines (n = 32, 53.3%). Median CT-SS value was 15 (IQR 11–19), and majority (n = 56, 93.3%) belonged to CO-RADS ≥ 4 .

CONCLUSION: Diagnosis of COVID-19 can be presumed in RT-PCR-negative suspected COVID-19 patients with moderate-to-severe disease, with marked rise of inflammatory markers and HRCT revealing typical findings of COVID-19 pneumonia.

Keywords:

Computed tomography, CO-RADS, coronavirus infection, polymerase chain reaction

Introduction

Early diagnosis of COVID-19 is the primary goal for the successful management of COVID-19 patients. Severe

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acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid detection by real-time reverse transcription polymerase chain reaction (RT–PCR) from upper and

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Received: 22-02-2022 Accepted: 17-05-2022 Published: 31-10-2022 lower respiratory tract specimens has remained the key test to diagnose the disease, though the problem lies in that the sensitivity of RT-PCR varies considerably for oropharyngeal (32%–48%) and nasopharyngeal samples (60%-70%). Sensitivity of RT-PCR depends on various factors, including the viral burden during specimen collection, sampling quality, and sensitivity of test kit.^[1-4] Moreover, RT-PCR test results can take even up to one week in different healthcare settings, subjected to the availability of testing resources. Patients with high clinical suspicion should undergo repeat testing which is time-consuming and critical for patients with moderate-to-severe disease.^[1,2]

Among the imaging techniques, sensitivity of chest X-ray in diagnosing COVID-19 even in moderate-to-severe forms of the disease is reported in up to 60%.^[5] Whereas, computed tomography (CT) scan of thorax has sensitivity and specificity in COVID-19 pneumonia of 60%-98% and 40%-60%, respectively with typical radiological findings.^[3,4,6,7] According to the COVID-19 Reporting and Data System (CO-RADS) developed by the Dutch Radiological Society based on CT scan findings and patient data, a cut point of 4 (CO-RADS 4) offers high sensitivity (80%–90%) and specificity (>90%) in diagnosing COVID-19.^[8] Another scoring system is CT scan severity score (CT-SS), which helps to assess disease severity in which cut-off value of 18 out of 25 (total score) can be a predictor of severe disease.[8-11] Few studies have even reported typical chest CT scan findings of COVID-19 in single or repeated RT-PCR-negative patients with high clinical suspicion of COVID-19 infection.^[12,13] COVID-19 patients requiring hospitalization commonly have altered levels of some laboratory markers like C-reactive protein (CRP), D-dimer, ferritin, lactate dehydrogenase (LDH), neutrophil and lymphocyte ratio (NLR), Interleukin-6, etc., Very few studies which compared these markers between confirmed COVID-19 patients and RT-PCR-negative patients with high clinical suspicion of COVID-19 disease have yielded varied results.^[3,13-17]

There is often dilemma in management when patients with COVID-19-like symptoms test negative in RT-PCR for one or more times. Such patients, when develop severe acute respiratory illness (SARI), are often stuck between a non-COVID diagnosis and an alternative confirmed diagnosis and raises multiple challenges in resource-poor settings. First, false negative results increase the chance of COVID-19 spread to the true negative patients staying in the same ward. Second, a false negative result changes the management decision that can adversely impact the treatment outcome.^[2] Therefore, for such patients, with moderate-to-severe symptoms requiring prompt and appropriate management, we can simultaneously look for the COVID-19 specific changes in other investigations. Only limited information is available on whether we can use the hematological and radiological features for the suspected COVID-19 patients with suggestive symptoms to improve clinical decision-making. Therefore, we conducted this study to evaluate the clinical, laboratory and high-resolution CT scan (HRCT) of thorax findings, among RT-PCR-negative COVID-19 suspects with moderate-to-severe symptoms.

Materials and Methods

Study design and setting

This hospital-based retrospective observational study was conducted on moderate-to-severe symptomatic COVID-19 suspects admitted to the SARI ward and the intensive care unit (ICU) of Deben Mahata Govt. Medical College and Hospital, Purulia, who were RT-PCR negative.

Study participants

The study sample included moderate-to-severe symptomatic COVID-19 suspects who were admitted to the SARI ward and ICU and were RT-PCR negative. Moderate and severe case definitions for COVID-19-suspected cases are given in Table 1a.^[18] The inclusion criteria were age \geq 12 yrs, moderate and severe symptoms, and RT-PCR negative in single or repeat tests. Patients were excluded if they met any of the following criteria: documented RT-PCR positive, RT-PCR negative mild symptoms, pre-existing lung disease including interstitial lung disease, pneumonia due to bacterial and tubercular etiology, non-infectious lung lesions in CT scan, e.g., lung malignancy, pneumothorax, pulmonary edema, and history of hemoptysis.

We recruited 60 participants for the study based on the admission rate and the feasibility after applying all of the inclusion and exclusion criteria. Study participants were admitted in hospital between March 2021 and June 2021. The study was conducted between July 2021 and December 2021 for a period of 6 months.

Severity	Defining criteria
Mild disease	Fever, cough, malaise, sore throat without shortness of breath
Moderate disease	Adolescent and adult with presence of fever ≥100°F with or without cough, sore throat, myalgia, difficulty in breathing plus any one of the following: 1. Respiratory rate >24/min 2. SpO ₂ <95% in room air
	 3. Altered sensorium: drowsiness / confusion / stupor 4. Infiltrates on CXR 5. Altered LFT/RFT.
Severe disease	Moderate disease with acute respiratory distress syndrome (ARDS) and/or sepsis with multi-organ dysfunction syndrome (MODS) and/or septic shock (SBP <90 or DBP <60 mmHg)

Data collection tool and technique

We collected data from the medical records section of the hospital using a predesigned data abstraction form. Two researchers (AB and AB) abstracted the records. When difficulty arose in data abstraction, all the researchers discussed and reached to a consensus.

Statistical analysis

We entered the data in Microsoft Excel and analyzed in Statistical Package for the Social Sciences (SPSS) version 21.0. We used descriptive statistics for the analysis. We expressed categorical variables in percentages, and the continuous variables through means and standard deviation (SD) or median and inter-quartile range (IQR) depending on the distribution pattern. To measure the correlation of CT-SS value with different parameters,

Table 1b: Clinical features of the participants

Table Tb. Offical features of the part	icipante
Clinical features	Value (%)
Age (years), mean (SD)	55.5 (14.1)
Male gender	43 (71.7)
History of pre-existing lung disease	0 (0)
Other comorbidities	19 (31.7)
Diabetes	8 (13.3)
Hypertension	7 (11.7)
Hypothyroidism	4 (6.7)
Chronic kidney disease	3 (5)
Dyslipidemia	3 (5)
Parkinsonism	1 (0.67)
HIV	1 (0.67)
Coronary artery disease	1 (0.67)
Takayasu disease	1 (0.67)
History of smoking	9 (15)
COVID-19 vaccination (partial/complete)	6 (10)
Duration of hospitalization, mean (SD)	10 (5)
Duration of fever, median (IQR)	5 (3-7)
Duration of cough, mean (SD)	7 (5-12)
Presented with	
Fever	60 (100)
Cough	54 (90)
Fatigue	54 (90)
Expectoration	4 (6.7)
Headache	5 (8.3)
Rhinorrhea	2 (3.3)
Anosmia	8 (13.3)
Sore throat	13 (21.7)
Dyspnoea	57 (95)
Diarrhea	9 (15)
Respiratory rate, median (IQR)	32 (29-38)
$SpO_2^{\%}$ in room air, median (IQR)	86 (80-90)
Maximum O ₂ flow/min, median (IQR)	10 (5.25-15)
Maximum temperature (°F), median (IQR)	100 (99-100.8)
SBP maximum (mm/Hg), Median (IQR)	130 (120-136)
SBP minimum, median (IQR)	113 (106-124)
Heart rate maximum, median (IQR)	120 (110-128)
Heart rate minimum, median (IQR)	96 (87-103)
ECG, ST-T changes (%)	10 (16.7)

we estimated Pearson's correlation efficient (r), and P value <0.05 was considered statistically significant.

Ethical consideration

The Institutional ethics committee (IEC) of the institution approved the study (Ethical code number: IEC/DMGMCH/2021/05 dated 14/06/2021).

Results

We recruited a total of 60 suspected COVID-19 patients after applying strict inclusion and exclusion criteria. Mean age of study participants was 55.5 ± 14.1 years and most of them were males (71.7%). They presented to the hospital with varying combination of COVID-19-like symptoms including fever in all cases, dyspnea (95%), cough (90%), fatigue (90%), etc., None of the participants had any known pre-existing lung diseases [Table 1b]. Overall, 19 patients (31.7%) had other comorbidities including diabetes, hypertension, and hypothyroidism either singly or in combination. Six patients were partial/completely vaccinated for COVID-19. The median SpO₂ level was 86% (IQR 80%–90%) and respiratory rate was 32/min (IQR 29-38). ST-T changes on ECG was present in 10 patients (16.7%). Median value of maximum oxygen requirement among study participants was 10 L/min (IQR 5.25-15). Recovery rate was found 95% (57 out of 60). Mean duration of hospital stay was 10 ± 5 days. Clinical profile of study subjects is described in Table 1b.

Among the laboratory parameters, some changes were seen frequently like rise of CRP, NLR, D-dimer, ferritin, LDH, total leucocyte count and decrease in lymphocyte count [Tables 2 and 3]. Chest radiograph detected mixed patterns of GGOs and consolidations (56.7%), only GGOs (23.3%), only consolidations (5%) and no changes in 9 (15%) patients.

As per HRCT thorax findings [Table 4], the median CT-SS value was 15 (IQR 11–19) and 20 patients (33.3%) had such value between 18–25. Common findings on HRCT

Table 2: Laboratory profile of the participants	Table 2:	Laboratory	profile	of the	participants
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Laboratory features	Obtained Values
Total leucocyte count (TLC),	10,800
median (IQR)	(1,900-14,000)/cumm
Lymphocytes, median (IQR)	1,498 (997-1,928)/cumm
Neutrophil: Lymphocytes, median (IQR)	5.6 (4-9.3)
Presence of Transaminitis (%)	32 (53.3)
Blood sugar, median (IQR)	158 (128-242) mg/dl
Creatinine, median (IQR)	1.1 (0.8-1.4) mg/dl
C-reactive protein, median (IQR))	46 (25-63) mg/L
D-dimer, median (IQR)	1.1 (0.6-1.5) µg/ml
Ferritin, median (IQR)	455 (300-849) ng/ml
LDH, median (IQR)	535 (405-830) IU/L

thorax were GGOs (mixed with consolidations 61.7%, only GGO 31.7%) with bilateral location and diffuse distribution, interlobar septal thickening (74.1%), vascular thickening (58.%), sub-pleural lines/bands (53.3%) and air bronchograms (53.3%) [Table 4, Figure 1]. Majority of patients (93.3%) belonged to CO-RADS 4 and above.

The CT-SS value in HRCT was significantly correlated with the duration of hospital stay (r = 0.37, P = 0.003), maximum oxygen requirement (r = 0.52, P < 0.001),

Blood parameter	n (%)	Normal reference value
Leukocytosis	30 (50)	4,000-11,000/cumm
Raised CRP	60 (100)	0-6 mg/L
Neutrophilia	36 (60)	1,500-8,000/cumm
Lymphopenia	15 (25)	1,000-4,800/cumm
Raised NLR	49 (81.7)	<3.13
Hyperglycemia	17 (28.3)	Max. blood glucose >200 mg/dl
Hypercreatinemia	12 (20)	0.7-1.4 mg/dl
Raised d-dimer	47 (78.3)	<0.5 µg/ml
Raised ferritin	46 (76.7)	25-280 ng/ml
Raised LDH	40 (66.7)	225-450 IU/L

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Table 4: HF	RCT thorax	findinas a	of the	participants
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Findings	Value (%)
Location	
Both lungs	60 (100)
Attenuation	
Ground glass opacity only	19 (31.7)
Consolidation	4 (6.7)
Mixed	37 (61.7)
Distribution	
Diffuse	36 (76.7)
Peripheral	14 (23.3)
Crazy paving	20 (33.3)
Interlobar septal thickening	43 (74.1)
Fibrotic changes	17 (29.3)
Air bronchogram	32 (53.3)
Bronchial wall thickening	0 (0)
Bronchiectasis	13 (21.7)
Mediastinal lymphadenopathy	0 (0)
Subpleural lines	32 (53.3)
Pleural thickening	4 (6.7)
Pleural effusion	3 (5)
Vascular thickening	35 (58.3)
Nodules	1 (1.7)
CT-SS score median (IQR)	15 (11-19)
Score 1-7	6 (10)
Score 8-17	34 (56.7)
Score 18-25	20 (33.3)
CO-RADS classification (Suspicion level)	
CO-RADS 1 (No)	0 (0)
CO-RADS 2 (Low)	0 (0)
CO-RADS 3 (Intermediate)	4 (6.7)
CO-RADS 4 (High)	4 (6.7)
CO-RADS 5 (Very high)	52 (86.6)

serum LDH (r = 0.26, P = 0.04), and CRP (r = 0.28, P = 0.03) [Table 5].

Discussion

In this hospital-based retrospective observational study, we described the clinical, laboratory and radiological profile of moderate-to-severe symptomatic COVID-19 suspects who were negative in RT-PCR testing for COVID-19 by single or multiple test results. These features are similar in respect to the patients often having a confirmed diagnosis of COVID-19 disease by positive RT-PCR report.

Among the study participants, fever, dyspnea, cough, fatigue were the most frequent presenting symptoms of varying duration. Most of the similar studies reported history of fever, cough, dyspnea in more than 60% of cases and less commonly nausea, headache, expectoration, chest pain which usually occur in less than 20% of patients.^[7,17] Clinical symptoms and signs like high respiratory rate, low oxygen saturation at presentation raised clinical suspicion of COVID-19 disease among our study.

Among laboratory parameters, common findings in COVID-19 RT-PCR-positive patients include raised CRP, D-dimer, ferritin, LDH levels with high NLR ratio and lymphopenia.^[13,15-17,19,20] We also found similar blood pictures in our patients in spite of being RT-PCR negative. Our results support findings of Orlachhio *et al.*^[13] which showed no significant difference in percentages alteration of lymphocytopenia, raised CRP, D-dimer and LDH in RT-PCR-positive versus -negative groups with COVID-19-like clinical features, though

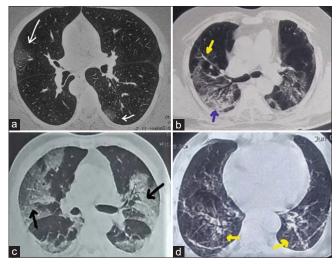


Figure 1: (a-d): Axial HRCT showing ground glass opacities (GGO) (white arrow in Figure 1a), GGO mixed with consolidations (blue arrow) and vascular thickening (yellow arrow) (Figure 1b), GGO with intra and interlobular septal thickening (crazy paving) (black arrows in Figure 1c), and bilateral lower lobe fibrosis with fibrotic bands (yellow arrow in Figure 1d)

Table 5:	Correlation	of C	T-SS	value	with	different
paramete	ers					

Parameters	Pearson's r	Р	
Age	0.060	0.649	
Hospital stay	0.373	0.003*	
Max. O ₂ requirement	0.526	<0.001*	
Ferritin	0.139	0.291	
LDH	0.262	0.043*	
NLR	0.053	0.69	
CRP	0.278	0.032*	
TLC	0.038	0.773	
D-dimer	0.107	0.414	
Blood sugar	0.217	0.095	

P<0.05 considered significant (*)

some studies have reported significantly higher levels for some of these parameters in RT-PCR proven COVID-19 patients after comparing these two groups.^[15-17] Chest radiographs (CXR) revealed ground glass opacities (GGO) and consolidation often with no findings in 15% of patients. Earlier studies also suggest similar findings and sensitivity of CXR to detect changes in COVID pneumonia is about 60%, which increases as the disease severity increases.^[1]

Evidence suggests that the predominant findings in HRCT among RT-PCR-positive COVID-19 pneumonia patients include bilateral location, diffuse opacities or peripheral opacities, isolated GGO or mixed with consolidations, crazy paving appearance, subpleural bands, fibrotic changes with bronchial wall thickening, bronchiectasis, pleural effusion, hilar lymphadenopathy, pulmonary nodules in very few patients.^[7,10] The HRCT findings in our study were similar to these findings. Orlachhio et al.^[13] reported no significant difference in the distribution of lung alterations and extent of lung damage between RT-PCR-positive and -negative patients. In our study, CT-SS of 1-7 (10%), 8-17 (56.7%), and 18-25 (33%) compared to 36.5%, 34.3%, 6.8% in Saeed *et al.*^[20] We had average CT-SS of 15 (range 11–19), while Chung et al.^[7] had average of 9.9 (range 0–19). Our study had higher values, most probably as we included only moderate-to-severe cases and cut-off value of 18 has been linked to severity as per previous studies.^[8–11] Most patients (86.6%) belonged to very high suspicion level (CO-RADS 5) for COVID-19 as per our finding. Zayed et al.^[8] reported average CO-RADS value of 4.8 in severe COVID-19 patients which is similar to us.

We tried to find if different patient parameters were related to CT-SS scores of respective patients and we found highly significant correlation of CT-SS values with maximum oxygen requirement (P < 0.001), which was obvious as higher scores indicate higher areas of lung damage with lower SpO₂ levels. It was also significantly correlated with duration of hospital stay and some

inflammatory biomarkers like CRP and LDH levels but D-dimer, ferritin, NLR had only non-significant correlation [Table 5]. Other studies also found significant correlation of CT-SS with some inflammatory markers, lymphopenia, maximum oxygen required, male gender, hospital stay in RT-PCR-proven and in non-proven cases too.^[10,13,20] Higher rise of inflammatory markers and increased time for recovery is very much associated with severe and critical patients which also is associated with higher CT-SS values.

So, different blood parameters with biomarkers of inflammation (CRP, D-dimer, ferritin, LDH) were commonly raised in our patients which are frequent findings in confirmed COVID-19 patients too, despite our patients being RT-PCR negative. Also, HRCT findings in our patients showed typical radiological changes usually found in COVID-19 patients, with maximum of them having CO-RADS score \geq 4 which is highly sensitive and specific for COVID-19. Very few studies are available worldwide, that too outside India, which describes HRCT thorax findings and inflammatory markers among moderate-to-severe symptomatic RT-PCR-negative COVID-19 suspects. Their results support our findings also.^[12,13,20]

Limitations and recommendation

This was a record-based study, and so, it was limited in abstracting all the information. Repeated RT-PCR testing was not always performed after initial negative report. Besides, the sample size was limited in our study. The finding must be used judiciously.

Conclusions

Patients with moderate-to-severe acute respiratory illness with negative RT-PCR report might have COVID-19 disease. In such patients, diagnosis of COVID-19 can be presumed from supportive clinical, laboratory and HRCT findings. A timely decision based on these findings can improve the clinical outcome of such patients.

Acknowledgement

We would like to thank our colleagues and staffs of Medical Record Section of our hospital for facilitating in data gathering.

Abbreviations

CO-RADS = COVID-19 Reporting and Data System CRP = C-reactive protein CT-SS = CT scan severity score CXR = Chest X-Ray GGO = Ground Glass Opacity HRCT = High resolution-computed tomography

IQR = Inter-quartile range

LDH = lactate dehydrogenase NLR = Neutrophil and lymphocyte ratio RT-PCR = Reverse transcription polymerase chain reaction (RT-PCR) SARI = Severe acute respiratory illness SD = Standard deviation

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Conflicts of interest

There are no conflicts of interest.

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