RELATION OF CHEST HRCT SCAN FINDINGS WITH HEMATOLOGICAL, BIOCHEMICAL, AND IMMUNOLOGICAL PARAMETERS OF HOSPITALIZED COVID-19 PATIENTS IN A TERTIARY HOSPITAL IN CHATTOGRAM CITY

By

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing the degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.

4. I/We have acknowledged all main sources of help.

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Approval

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Of Fall 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters in Biotechnology on [23-03-2023].

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Ethics Statement

This material is an original work, which has not been previously published elsewhere. It is my own research and analysis in a truthful and complete manner. The paper properly credits all the sources used (correct citation).

Abstract

The chest HRCT scan can be used as a diagnostic tool for COVID-19 disease in cases of clinical suspicion, false-negative RT-PCR, or unavailability of the test. The study aimed to investigate the relation of HRCT findings (1) Normal, (2) Ground Glass Opacity (GGO), and (3) GGO plus Consolidation (GGO+Co) with hematological, biochemical, immunological parameters, and clinical manifestations of COVID-19 patients. As such, oone hundred and thirty-five COVID-19 RT-PCR-confirmed patients were enrolled in this study from a dedicated COVID-19 ward in a tertiary care hospitals in Chattogram city. Chest HRCT scans were analyzed for all enrolled patients. D-dimer, CRP, procalcitonin, IL-6, and total count/TC, differential count/DC, and Platelet count were analyzed by automated biochemistry and hematology analyzer. Among the participants, 62.7% were male and 37.3% female with age for both genders were 49 years. Based on HRCT density, normal, GGO, and GGO+Co cases were identified in 36%, 21%, and 43% of the patients, respectively. Lung infections were not detected in the normal group but were present in the GGO and GGO+Co groups. Mild (75.86%), moderate (13.79%), and severe (10.34%) cases were found in the GGO,group, and they were 24.13%, 50%, and 24.13% respectively in GGO+Co groups. Critical cases (1.72%) were found only in the GGO+Co group. Clinical manifestations were recorded in three groups of COVID-19 patients where the fever was the most predominant symptom in the Normal (91.67%), GGO (92.85%), and GGO+Co (96.55%) groups, followed by cough in Normal (68.08%), GGO (71.42%), and GGO+Co (72.41%). In the Normal patients group, Mild, Moderate, and severe oxygen saturation (SpO_2) levels in N=47(97.92%, N=1(2.08%), and N=0 (0%) respectively. SpO_2 levels, Mild 93.10% (N=27), Moderate 6.89 %(N=2), and Severe 0 %(N=0) had detected in GGO patients groups. On the other hand, SpO_2 level N=39/67.24% (Mild), N=1/1.72 %(Moderate), and only N=18/31.03 %(Severe) cases were found in GGO+Co patients groups. GGO+Co group had higher levels of CRP (82.37 mg/L), D-dimer (0.97 µg/ml), and PCT (0.68 ng/mL) compared to normal cases with CRP (13.22 mg/L), D-dimer(0.44 µg/ml), and PCT (0.14 ng/mL), and GGO cases had levels of CRP (25.88 mg/L), D-dimer (0.55 µg/ml), and PCT (0.22 ng/mL). Significant p-values (GGO+Co vs normal) were found for CRP (p=0.000000077*) and PCT (p=0.000000077*). Differences in hematological features were also observed, with higher counts neutrophils (72.45%), and platelets (231.92 x 10^9/L) in the GGO+Co group compared to the normal and GGO cases. Il-6 mean levels were 52.34pg/ML, 80.6 pg/ML, and 105.78 pg/ML for normal, GGO, and GGO+Co groups, respectively. In suspected COVID-19 RT-PCR false negative cases, chest HRCT can be used as an alternative test, and its laboratory findings (CRP, PCT, D-dimer, Total & differential Count of WBC, Platelet) can be taken as prognostic markers for the treatment of COVID-19 to reduce the mortality rate.

Keywords: RT-PCR; HRCT; Normal chest CT; GGO; GGO+CO; CRP; D-dimer; PCT; IL-6; Clinical manifestation;SpO2; and Hematological Parameters (TC, DC, Platelet).

Dedicated to my beloved family

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I would like to acknowledge my respected supervisor, Professor Dr. Mahboob Hossain, joint- supervisor Prof. Dr. Muhammad Akram Hossain, and everyone who has helped at any point during my thesis

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List of Acronyms

RT-PCR	Real Time PCR
HRCT	High Resolution of Computed Tomography
COVID-19	Coronavirus disease 2019
CRP	C Reactive Protein
CT	Cycle Threshold
SARS CoV-2	Severe Acute Respiratory Syndrome Corona Virus 2
PCT	Procalcitonin
CBC	Complete Blood Count
TC	Total Count
DC	Differential Count
GGO	Ground Glass Opacity
GGO+Co	GGO plus Consolidation
SpO2	Oxygen Saturation
IL-6	Interleukin 6
WHO	World Health Organization
CDC	Center of Disease Control
PC	Positive Control
NC	Negative Control
WBC	White Blood Cells

Chapter 1: Introduction

1.1 Background

Coronavirus disease 2019 (COVID-19), a highly infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, Hubei Province, China, and rapidly spreads to other countries (Zhu et al., 2020). World Health Organization (WHO) declared this ongoing outbreak as a global public health emergency on January 30, 2020, and raised the risk of COVID-19 to very high at the global level on February 28, 2020 (Coronavirus Disease (COVID-19) – World Health Organization, 2020). A total of 559,469,605 COVID-19 cases with 6,361,157 deaths were confirmed as of July 18, 2022 (Coronavirus Disease (COVID-19) – World Health Organization, 2022). As the mechanism of action, SARS-CoV-2 was reported to target angiotensin-converting enzyme-2 (ACE2) as the cell receptor in humans, firstly causing pulmonary interstitial damage and subsequently with parenchymal changes (Xu et al., 2020).

For the diagnosis of COVID-19, real-time reverse transcription polymerase chain reaction (RT-PCR) of viral nucleic acid is regarded as the gold standard; however, a noncontract high-resolution computed tomography (HRCT) along with detection of different biochemical, immunological, and hematological parameters also plays a pivotal and essential role in the early disease detection, particularly in patients with false-negative RT-PCR results, as well as in managing and monitoring the course of disease (Liu et al., 2020).

HRCT images of the chest could manifest different imaging features or patterns in COVID-19 patients with a different time courses and disease severity (Shi et al., 2020, Song et al., 2020&Pan et al., 2020). Ground glass opacities (GGO) were defined as hazy areas with slightly increased density in lungs without obscuration of bronchial and vascular margins, which may be caused by the partial

displacement of air due to partial filling of airspaces or interstitial thickening (Hansell et al., 2008). In patients with COVID-19, unilateral or bilateral GGO with a peripheral lung and subpleural distribution are commonly encountered (Pan et al., 2020&Ng et al., 2020). GGO is the most common imaging finding with an occurrence rate of up to 98% (Li et al., 2020). On the other hand, consolidation refers to alveolar air being replaced by pathological fluids, cells, or tissues, manifested by an increase in pulmonary parenchymal density that obscures the margins of underlying vessels and airway walls [9]. Multifocal, patchy, or segmental consolidation, distributed in subpleural areas or along bronchovascular bundles, is usually presented in COVID-19 patients with an occurrence rate of 2~64% (Bernheim et al., 2020&Wu et al., 2020). Bilateral distribution of ground glass opacities (GGO) with or without consolidation in posterior and peripheral lungs was the cardinal hallmark of COVID-19 (Wang et al., 2020 &Chung et al., 2020). Prognosis can also be affected by the severity of the disease in critically ill patients allowing appropriate selection of early involvement in the intensive care (Chung et al., 2020&Leonardi et al., 2020).

The use of routine blood investigation parameters as a marker of disease severity will result in improved clinical awareness to identify the target patients at higher risk (Huang et al., 2020). A simple blood test may have an important role in the diagnosis & monitoring of disease condition, as the test provides information on the inflammatory process including leucocyte count and other characteristics such as neutrophil- or lymphocyte-dominance, neutrophil-lymphocyte ratio (N/L ratio), C-reactive protein (CRP) as inflammation marker, and the disease severity. An analysis of blood test results may provide information in terms of the nature of pneumonia, where the physician can determine the etiology of the disease (Bekdas M. et al.,2014). As a marker of an inflammatory process, complete blood count (CBC) including platelet count (PLT), neutrophils, lymphocyte, and monocyte count. Neutrophils are important components of the immune system. Considering the prognostic

indicator of COVID-19-positive patients, the use of circulating biomarkers representing inflammation and the immune system may be of great importance.

In recent systematic research and meta-analysis reviews, low platelet count, elevated C-reactive protein (CRP), and LDH were found to be associated with mortality in COVID-19 patients (Qiu et al., 2020). Another study argued that procalcitonin (PCT), CRP, and LDH levels demonstrated significant elevations in a pooled laboratory analysis of children with mild and severe COVID-19 (Henry et al., 2020). In a different study, laboratory markers especially low lymphocyte count, ferritin, D-dimer, CRP, cardiac troponin, and LDH were significant parameters with predictive value in patients with severe and mild COVID-19 disease (Velavan & Meyer, 2020). CRP and interleukin 6 (IL-6) levels increased in severe stages of the disease (Liu et al., 2020), and CRP >41.8 mg/L may be an independent risk factor for progression in early-stage COVID-19 patients. Elevated CRP accompanies lung lesions and therefore reflects the severity of the disease (Ahnach et al., 2020). Also, the elevated D-dimer, prolonged prothrombin time (PT), and activated partial thromboplastin time (APTT) in addition to the increase in CRP, IL-6, and LDH levels might result in in disseminated intravascular coagulation (Terpos et al., 2020). Similarly, many recent studies demonstrated neutrophil/lymphocyte ratio as an independent risk factor for severe COVID-19 disease (Liu et al., 2020).

1.2 Morphology of SARS-CoV-2 Virus

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) the coronavirus responsible for COVID-19 has been studied using electron microscopy, which has revealed its unique morphology (Figure-1). Virus particle sizes ranging from 70 to 90 nm have been observed in various intracellular organelles, specifically in vesicles (Park et al., 2020). It is speculated that the structure of SARS-CoV-2 is identical to that of SARS-CoV due to their high sequence similarity (Kumar et al., 2020). The virus's surface protein spike, membrane, and envelope are embedded in a lipid bilayer derived from the

host's membrane, which encloses the helical nucleocapsid containing the viral RNA (Finlay et al., 2004). The structures of the spike (Yan et al., 2020) and protease (Zhang et al., 2020) of SARS-CoV-2 have been resolved, presenting an opportunity to develop new drug treatments for COVID-19.

According to (Chen et al., 2020), the viral envelope of SARS-CoV-2 is constructed of three proteins: the S-spike protein, which creates peplomers and imparts the virus its distinctive crown shape; the M-membrane protein; and the E-envelope protein, which provide the ring structure. In addition, there is a fourth protein, the N-nucleocapsid protein, a phosphoprotein that functions as a structural component of the nucleocapsid (Wu et al., 2020). The S protein, which belongs to the group I fusion glycoproteins, has a homotrimeric structure with a single upper and two lower conformations (Walls et al., 2020). The amino acid sequence identity between the S protein of SARS-CoV-1 and SARS-CoV-2 is approximately 75.5% (Zheng et al., 2020). The spike consists of two subunits, the N-terminal S1 and the C-terminal S2, which are accountable for association with the host cell and virion endocytosis, respectively. A 4-amino-acid region separating them is implicated in furin protein cleavage during biosynthesis, distinguishing SARS-CoV-2 from SARS-CoV-1 (Gussow et al., 2020).

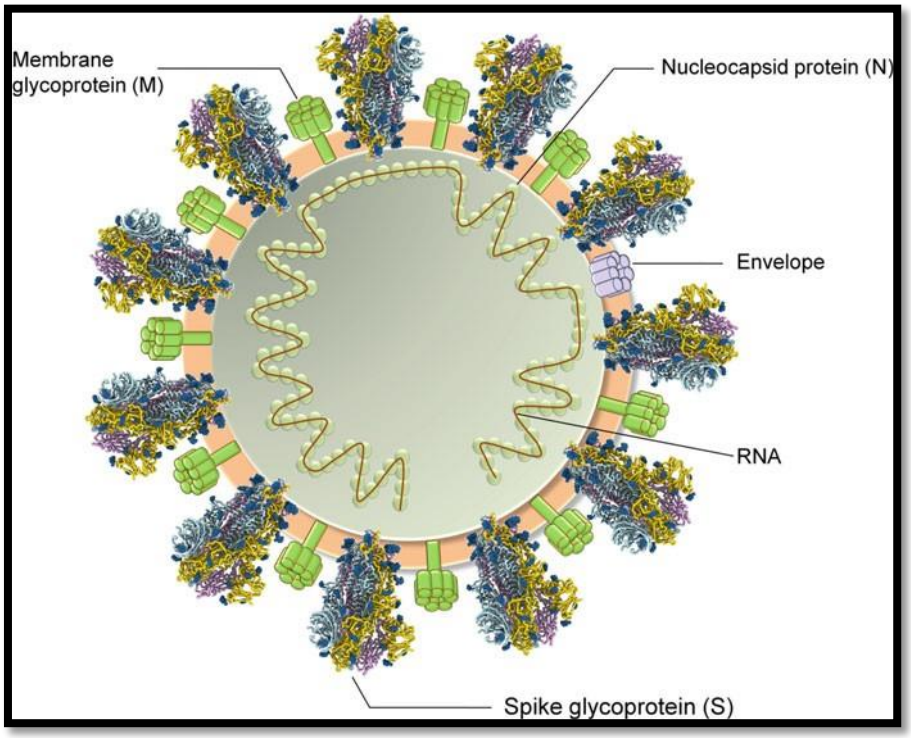

Figure1: The structure of SARS-CoV-2. The virus consists of surface viral proteins, spike glycoprotein (S), membrane glycoprotein (M), and Nucleocapsid protein (N) of SARS-CoV-2 (Kumar et al., 2020).

1.3 Genomic structure of SARS-CoV-2

The coronavirus genome ranges from 26 to 32 kb and contains 6-11 open reading frames (ORFs) that encode 9680 amino acid polyproteins. The first ORF, which makes up about 67% of the genome, encodes 16 nonstructural proteins (nsps), while the other ORFs encode accessory and structural proteins. SARS-CoV-2's genome does not have the hemagglutinin-esterase gene, but it has two untranslated regions (UTRs) flanking its 5' end of 265 nucleotides and its 3' end of 358 nucleotides. Comparing SARS-CoV-2 and SARS-CoV, there is no significant difference in ORFs and nsps. SARS-CoV-2's nsps include two viral cysteine proteases (papain-like protease (nsp3) and chymotrypsin-like, 3C-like, or main protease (nsp5), RNA-dependent RNA polymerase (nsp12),

helicase (nsp13), and others that are likely involved in SARS-CoV-2 transcription and replication (Chan et al. 2020).Along with nsps, there are four major structural proteins: spike surface glycoprotein (S), membrane, nucleocapsid protein (N), envelope (E), and accessory proteins encoded by ORFs. The N-terminal glycosylated ectodomain appears at the N-terminal end of the M protein, which consists of three transmembrane domains (TM) and a long C-terminal CT domain (Figure-2)

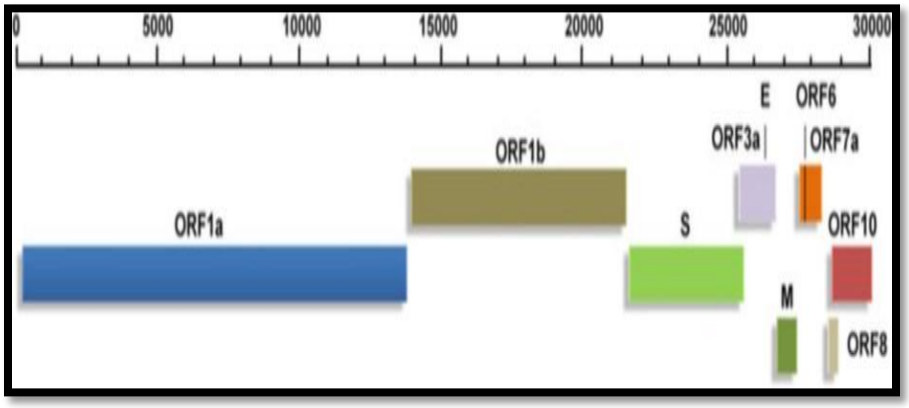

Figure 2: Genomic structure of SARS-CoV-2. The genome encodes for four major structural proteins including spike surface glycoprotein (S), membrane protein (M), nucleocapsid protein (N), and envelope (E). Accessory proteins are encoded by open reading frames (ORFs) (Kumar et al., 2020).

The M and E proteins play a crucial role in the assembly, budding, and morphogenesis of the virus, while the S glycoprotein is a fusion viral protein that consists of two subunits, S1 and S2. The S1 subunit has a signal peptide, a receptor-binding domain (RBD), and an N-terminal domain (NTD), and shares 70% sequence identity with bat SARS-like CoVs and human SARS-CoV(Walls et al. 2020). Although the external subdomain, which is primarily responsible for interaction with the ACE2 receptor, showed differences. Researchers have cloned, expressed, and crystallized the ectodomain of spike protein (1–1208 amino acid residues) to analyze the structure of the spike

glycoprotein of SARS-CoV-2. The structure of the spike glycoprotein of SARS-CoV-2 is similar to that of SARS-CoV with an RMSD of 3.8 Å. The receptor-binding region (RBD) showed the highest structural divergence (Wrapp et al., 2020). The S2 subunit, which shares 99% sequence identity with bat SARS-like CoVs and human SARS-CoV, consists of two heptad repeat regions (HR-N and HR-C) that form coiled-coil structures surrounded by the protein ectodomain. The S protein contains a furin cleavage site (PRRARS'V) at the interface between S1 and S2 subunits that is processed during the biogenesis (Coutard et al.,2020).

1.3 How SARS-CoV-2 Enters and Replicates in Host Cells

Coronaviruses enter host cells by binding their spike glycoprotein to a cellular receptor and the priming of S protein by host cell proteases.

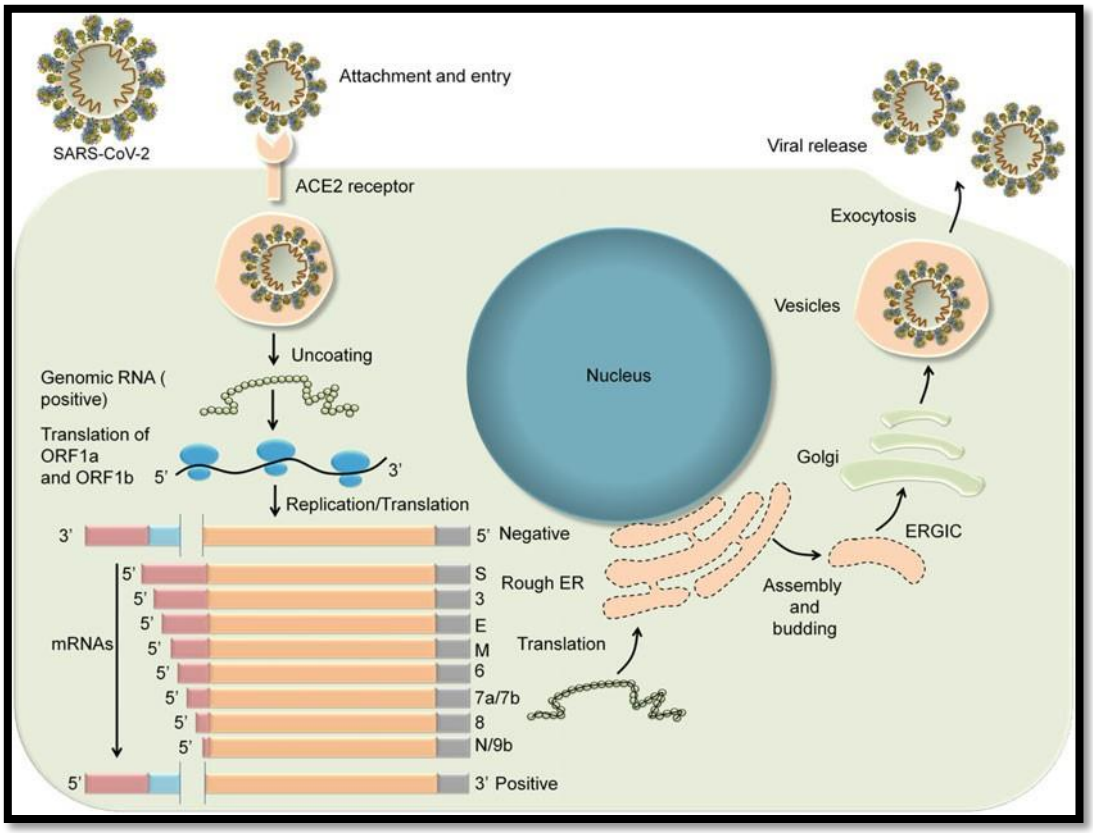

Figure 3: Entry and replication of SARS-CoV-2 Virus in host cells (Coronavirus Disease 2019 (COVID-19), ISBN: 978-981-15-4813-0).

SARS-CoV-2, like SARS-CoV, uses the ACE2 receptor for internalization and TMPRSS2 serine proteases for S protein priming, resulting in the extrapulmonary spread of the virus due to the widespread tissue expression of the ACE2 receptor.

Studies suggest that the spike protein of SARS-CoV-2 has a higher affinity than that of SARS-CoV **(**Hoffmann et al. 2020& Wrapp et al. 2020). The interaction between the spike protein and the ACE2 receptor triggers a series of structural alterations in the spike protein, resulting in the fusion of the viral envelope protein with the host cell membrane. This fusion allows the virus to enter the host cell via the endosomal pathway, releasing viral RNA into the host cytoplasm. The viral RNA undergoes translation, generating replicase polyproteins pp1a and pp1b, which are further cleaved by virus-encoded proteinases into smaller proteins (Coutard et al., 2020; Matsuyama and Taguchi, 2009).

During replication, ribosomal frame shifting generates both genomic and multiple copies of subgenomic RNA species that encode for relevant viral proteins. Virion assembly occurs via the interaction of viral RNA and protein at the endoplasmic reticulum (ER) and Golgi complex, and then they are released from cells via vesicles(Figure-3), (Hoffmann et al.,2020).

Chapter 2: Literature Review

2.1 Clinical Manifestation of COVID-19

Severe Acute Respiratory Syndrome (SARS) was initiated by zoonotic transmission of a novel coronavirus (likely from bats via palm civets) in markets in Guangdong Province, China. Middle East Respiratory Syndrome (MERS) was also traced to the zoonotic transmission of a novel coronavirus (likely from bats via dromedary camels) in Saudi Arabia. Finally, SARS CoV-2 which first appeared in 2019 in Wuhan, China, is also suspected of animal origin. All 3 viral infections commonly present with fever, and cough, which frequently lead to lower respiratory tract disease with poor clinical outcomes associated with older age and underlying health conditions. The clinical manifestation of COVID-19 infection is fever, nasal congestion, dry cough,sore throat, shortness of breath, Oxygen saturation (SpO2) level and diarrhea (Grudlewska-Buda et al., 2021).

2.2 Characteristics and Diagnosis of COVID-19

(Wu, Z., & McGoogan, J. M.,2020) et al., Summarize a report of 72314 cases from the Chinese center for disease control and prevention where the discussion was held about the Characteristics and important lessons from the corona virus disease 2019 (COVID-19) outbreak in China. According to them, the COVID-19 outbreak is both similar and different to the prior severe acute respiratory syndrome (SARS; 2002-2003) and Middle East respiratory syndrome (MERS; 2012-ongoing) outbreaks. Confirmation of infection requires nucleic acid testing of respiratory tract samples (e.g., throat swabs), but clinical diagnosis may be made based on clinical symptoms, exposures, and chest imaging. Supportive care for patients is typically the standard protocol because no specific effective antiviral therapies have been identified. Most of the patients were 30 to 79 years of age (87%), 1% were aged 9 years or younger, 1% were aged 10 to 19 years, and 3% were aged 80 years or older.

Most cases were classified as mild. However, 14% were severe and 5% were critical. The overall case-fatality rate (CFR) was 2.3%. CFR was elevated among those with preexisting comorbid conditions—10.5% for cardiovascular disease, 7.3% for diabetes, 6.3% for chronic respiratory disease, 6.0% for hypertension, and 5.6% for cancer.

(Lu R, Zhao X, Li J, et al., 2019) discusses the epidemiology of the 2019 novel corona virus and genomically characterizes for virus origins. They did next-generation sequencing of samples from bronchoalveolar lavage fluid and came tothe conclusion that 2019-nCoV was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45, and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, eastern China, but were more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%). The severe prognosis of COVID-19 has been associated with co-morbidities including diabetes, hypertension, cardiovascular disease, chronic obstructive pulmonary diseases, malignancy, and chronic liver disease. Although most COVID-19-infected patients are thought to be recovered after a few days of infections, patients with various chronic diseases may have fatal outcomes.

2.3 Chest imaging for the diagnosis of COVID-19

(Kashyape R et al., 2021) find out the utility of HRCT in the initial diagnosis of COVID-19 pneumonia in India. They proposed that HRCT might be an excellent adjunct for initial diagnosis of COVID-19 pneumonia in both symptomatic and asymptomatic individuals in addition to the role of prognostic indicator for COVID-19 pneumonia as they found 85% positive predictive value for HRCT and 73% sensitivity for all the patients. Overall, accuracy was 68%.

2.4 Laboratory Investigations of COVID-19 Detected Patients.

(Dubey D. B. et al., 2021) done a study from a tertiary hospital in North India and defined the indicator of severally ill versus mild Covid-19 patients. They assessed the hematological and serum biochemistry parameters and correlated them with the presenting symptoms and severity of the disease which could help predict the need for intensive care unit (ICU) care. The mean differences of TLC, Neutrophil% (N%), Lymphocyte% (L%), and Monocyte (M%) were significantly different between mild and moderate symptomatic cases ($p = 0.030$, $p = 0.002$, $p = 0.004$ & $p = 0.003$). Comparison of the mild vs. severely ill cases showed a significant difference in urea, fibrinogen, and procalcitonin (PCT) levels ($p = 0.005$, $p = 0.000$ & $p = 0.048$) respectively.

(Orlacchio A et al., 2021) correlates between chest-CT and laboratory parameters in SARS-CoV-2 pneumonia In Italy. They investigated the relationship between damaged lungs assessed by chest computed tomography (CT) scan and laboratory biochemical parameters to find other diagnostic tools. They found that Lymphocytopenia, C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, and fibrinogen had increased levels and occurred in most of the patients without statistically significant differences between the 2 groups and thus CT scan was suggest for COVID-19 diagnosis. The volume of lung damage was strongly associated with altered laboratory test results, even for the patients with negative RT-PCR test, and came, in conclusion, was that the decreased number of lymphocytes and the increased levels of CRP, LDH, D-dimer, and fibrinogen levels were associated with SARS-CoV 2 related pneumonia.

(Bilgir et al., 2021) retrospectively analyzed the roles of certain biochemical and hematological parameters in predicting mortality and ICU admission of COVID-19 patients. They found that the red cell distribution width, ferritin, lactate dehydrogenase, D-dimer, C-reactive protein, prothrombin time, and creatinine levels were the most significant parameters. They found that these parameters

weresignificant for predicting not only intensive care unit admission but also the mortality of the patients admitted to the intensive care unit.

It is evident from the studies in the literature that various parameters are used in determining severe and mild COVID-19 infections. However, the parameters that are effective in determining biomarker-based mortality and severity statuses of these patients should be predicted. For these reasons, the study aimed to retrospectively analyze the roles of certain hematological, immunological, and biochemical parameters in predicting mortality and severity statuses in terms of mild, moderate, and critical clinical manifestations and thus establish a biomarker-based prediction model of the patients who are confirmed to have COVID-19 disease by HRCT/RT-PCR. To our knowledge, thisis the first comprehensive study to describe a prediction model throughthe correlation of chest CT severity scores and the clinical picture with biochemical, immunological, and hematological parameters of COVID-19-positive patients in Bangladesh.

2.5 Objectives of the study

a) To differentiate High-Resolution Computed Tomography (HRCT) findings, including (1) normal, (2) Ground Glass Opacity (GGO), and (3) GGO plus consolidation in COVID-19 hospitalized patients.

b) To investigate the level of infection among the three different patient groups: normal, GGO, and GGO with consolidation.

c) To identify the clinical manifestation spectrum (fever, cough, loose motion, shortness of breath, respiratory distress, sore throat, and loss of smell and taste) between the three patient groups: normal, GGO, and GGO with consolidation.

d) The purpose is to investigate the condition-based oxygen saturation (SpO_2) in different patient groups.

e) To determine the relationships between HRCT and biochemical (D-dimer, CRP, procalcitonin), immunological (IL-6) parameters, and hematological (total count (TC), differential count (DC), platelet count) parameters.

f) The goal is to develop a panel of prognostic markers for COVID-19.

Chapter 3: Methods and Materials

3.1 Place of the study

The study was conducted in the Molecular Biology laboratory data facility from Apollo Imperial Hospital Limited (AIH), Chattogram Bangladesh.

3.2 Permission of the Study

Before starting the lab data collection, permission was taken from the Head of the Department of Molecular Biology at Apollo Imperial Hospital Limited (AIH).

3.3 Types of Study

This is a Retrospective study.

3.4 Study participants

The patients (N=135) enrolled in the study were admitted to the COVID-19 ward of the hospital. The patient's ages were between 7 to 85 years. The emergency doctor suspected the COVID-19 case clinically, then sent the sample to lab for RT-PCR and transferred the patient to an isolation cabin. The SARS CoV-2 RT-PCR Positive patients were transferred to the COVID ward and the negative patients were excluded from the study.

3.5 Inclusion Criteria

COVID-19 Infection:

- Subjective Fever
- Cough
- Shortness of Breath
- Loss of smell
- Sore throat

3.6 Period of the Study

The study was carried out from June 2021 to July 2021

3.7 Overviewof the Study

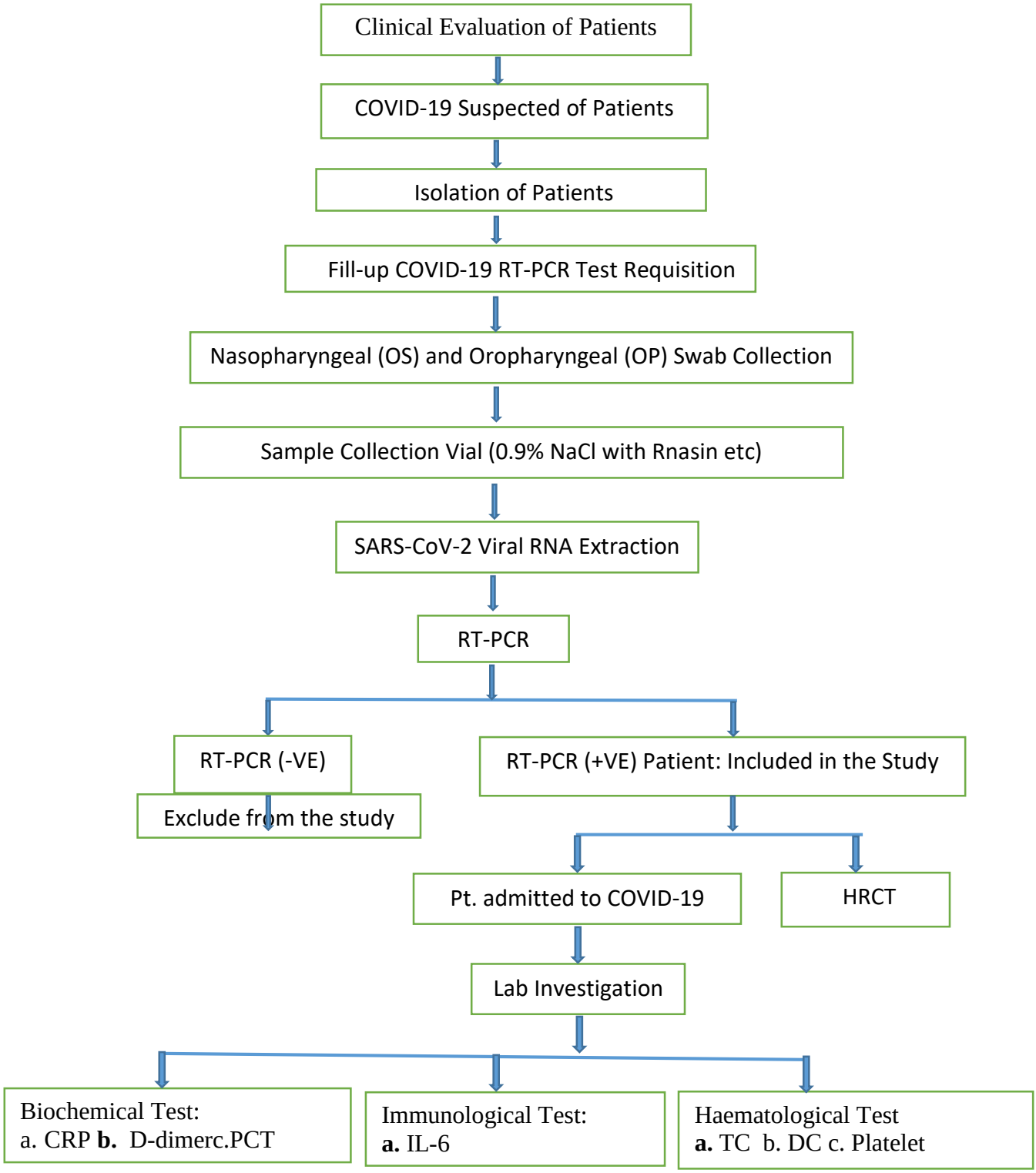

Figure 4: Overview of the study plan

3.8. Sample Collection Procedure of SARS-CoV2 RT-PCR

3.8.1 Equipment and Materials for sample collection

Equipment

a) Sample collection booth

b) Table for specimen cool box and materials

c) Patient's specimen collection chair

Materials/Reagents

a) 2 ml DNase Rnase Free sample storage vial containing 0.9% normal saline, Rnasin etc.

b) PPE (Mask,Gown,Gloves,Shoe cover)

c) Flocked swab/ Swab stick

d) Registration Barcode Sticker

e) Specimen Cool Box with rack

f) Hexisol (Hand Rub)

g) 0.1% Sodium Hypochlorite

h) Towel Tissue

Sample- Nasopharyngeal (NS) and Oropharyngeal (OP) Swab

3.8.2 Nasopharyngeal Swab (NS) Collection Procedure

1. Sample collection area or work space was cleaned and disinfected by 1% sodium hypochlorite (After collection of each sample)
2. The COVID-19 RT-PCR Fill-up form was taken from the patient's attendant
3. Label the specimen collection vial, containing the patient's name, and age with Software ID number and barcode /sample ID by the Registration officer

4. The dedicated phlebotomist/Nurse wore appropriate personal protective equipment (PPE) as per WHO guidelines before starting the procedure
5. The specimen was collected in the collection booth
6. Two millilitersample collection vial was taken with the attached barcode
7. The sample collection procedure was delivered to patients to ensure full cooperation.
8. The specimen was collected under good illumination.
9. A Nylon Flock swab stick was taken
10. The patient's head was tilted backward gently and the chin kept steady
11. The swab was inserted into the nostril (1-2 cm) parallel to the palate until it is obstructed by turbinate

14. The swab was held in that position for a few seconds and then withdrawn slowly in a firmly rotating motion (5 times clockwise and 5 times anticlockwise)

15. If a deviated septum or blockage creates trouble in obtaining the specimen from one nostril, the same swab was used to obtain the specimen from the other nostril

16. The applicator stick was broken off at the indicated mark (if provided) or below the level of the tube opening
17. The screw caps of the tube was closed tightly

3.8.3 Oropharyngeal Swab (OP) Collection Procedure

1. The patient’s head was gently tilted backward.
2. The chin was steady
3. The patient was asked to open his/her mouth and a disposable tongue depressor was used to hold the tongue well

4. A sterile swab namely a Flocked stick was inserted into the patient's mouth
5. Swab was inserted into the patient's mouth, both the tonsils and the posterior pharynx vigorously in a rotating motion, till the patient starts to gag. Touching the tongue, teeth, and gums wereavoided while removing the swab
6. Then the swab was placed in the labeled tube containing 0.9% Normal saline with Rnasin, stored in a specimen cool box and finally sent to the lab within 4 hours
7. The screw caps of the tube were tightly closed
8. The applicator stick was broken off at the indicated mark (if provided) or at below the level of the tube opening

3.8.4 Sample processing at the collection site

1. A unique patient and specimen ID was written/ pasted on each sample collection vial
2. After sample collection the vial was kept in a double-lock poly or zipper bag and then stored in the cool box immediately after collection

3.9 Transportation of SARS CoV-2 RT-PCR samples

1. Specimen: For initial testing of SARS-COV-2 RT Real-time PCR test both the nasopharyngeal (NP) and Oropharyngeal (OP) swabs are acceptable, a preferable swab is an NP swab
2. Transport: After the SARS-COV-2 sample collection the specimen was immediately kept in a cool box with an ice gel pack for maintaining 2-8^{0}C. The specimen was transported to the laboratory within 4 hours

3.Samples were kept in proper standing position in appropriate test tube rack

4. The sample collection tube was kept in direct contact with frozen gel packs

3.10 Submission of SARS CoV-2 RT-PCR samples to Lab

Collected specimens were sent to laboratories along with the COVID-19 test sample collection sheet and SARS-CoV-2 RT-PCR examination form by the assigned PAC (Patients Care Attendance).

3.11 RNA Extraction of COVID-19 sample by SANSURE sample release reagent

3.11.1 Method Principle

Sample release reagents have been developed based on the Sansure one-tube fast-release technology platform. The nucleic acid release kit patented by Sansure Biotech can rapidly lyse pathogens at room temperature, there is no need for any additional steps like heating, centrifuging, or replacing tubes. The sample DNA/RNA can be extracted quickly through simple procedures.

The Sample Release Reagent is used for the pretreatment of nucleic acids, to release the nucleic acids from specimens, then the released nucleic acids can be used for SARS-CoV-2 Reverse Transcriptase Real-Time PCR (RT-PCR).

3.11.2 Equipment and Materials for RNA Extraction

Equipment

a) BSL 2 Cabinet (ALS, Model: VBH 36C2,Manufacturer-Angetantoni Lifescience,Italy)

b) -20^{0}C Lab Benchtop Freeze-for sample storage (Lec Medical,Model:LSFSF39)

c) Microcentrifuge (Thermo scientific, Model- Micro CL 17R)

d) 2-8^{0}C Refrigerator for reagent and chemical storage (AUCMA,MODEL:SC37)

Materials/Reagents

a) 1.5 ml Microcentrifuge tube

b) 100-1000 µL Pipettes (ratio pipette)

c) 20-200 µL Pipettes

d) 2-20 µL Pipettes

e) 0.2-2 µL Pipettes

f) 70% Ethanol

g) Rnase Free Filter tips 2-20 µl

h) Rnase Free Filter tips 200 µl

i) Rnase Free Filter tips 1000 µl

j) CD Marker

k) Powder free sterile gloves

l) Towel tissue

m) RNA Extraction Worksheet

n) Ball pen

o) 1.5 ml Microcentrifuge rack (8X12)

p) 0.1% NaClO (Sodium Hypochloride)

q) 1000 ml discard beaker

r) 1.5 ml Microcentrifuge storage Box (8X12)

Reagent

Table 1: Ingredients, preservation and quantity of COVID-19 RT-PCR sample lysis solution

Name of Reagent (Manufacturer-Sansure Biotech)	**Quantity (24 Test)**	**Ingredients**	**Storage**
Sample Release Reagent	1.0mL/tube x 1 tube	Lysis buffer(SO3)	2-8ºC

3.11.3 Procedure of RNA Extraction

1.The UV light was kept on in Biosafety cabinet (BSC) II for 15 minutes before starting the RNA extraction

2.Biosafety cabinet was cleaned with the 70% ethanol

3.Disinfectants solution**,** 0.1% NaClO was prepared from stock concentration

4. The beaker was filled with 0.1% sodium hypochloride disinfectant to discard the used filter tips

5. Sample Release reagent was taken out from the refrigerator to thaw and then mixed by gentle pipetting

6. 20 µl sample release reagent was taken into a 1.5mL nuclease-free microcentrifuge tube

7. The sample vial was briefly vortexes and 20µl of the sample was taken into the same 1.5mL micro centrifuge

8. Pipetting those 3-5 times to completely mix the liquid

9. Centrifugation was done at 2000 rpm for 1 minutes

10. Then micro centrifuge tube was incubated at room temperature for 10 minutes

11. After incubation the RNA was ready for PCR

3.12 The procedure of SARS-COV-2 RNA detection by RT-Real time PCR (Qualitative)

3.12.1 Safety

a) Infection and Prevention control (IPC) of COVID-19 protocol was maintained during the test

b) Personal Protective Equipment (PPE) e.g.- N95 respiratory mask, goggles, face shield, and gloves were used and hand hygiene was practiced during the RT-PCR

c) Good laboratory Practiced (GLP) was while handling laboratory reagents

d) RNA extraction,Master Mix preparation, Template addition, and Amplification were done in a separate room

e) RNA Extraction-The specimen cool box was opened in the BSL-2 cabinet and RNA extraction is done manually following the protocol of the manufacturer

f) PCR Preparation- Master Mix was done in laminar airflow

g) The addition of Extracted RNA in the master mixture was done inside the laminar airflow cabinet.

h) The bench and Machine Monitor were cleaned with 70% Ethanol

i) The used PCR Reaction tube was discarded into 0.1% Hypochlorite solution

j) After the RT-PCR test, the machine was covered

k) Before leaving the lab

   i. The bench was clean with 70% Ethanol
   ii. Doffing (Removing of Gown, mask, and gloves) was done
   iii. All lights humidifier and the air conditioner was switched off

3.12.2 Specimen Used and Storage

a) **Sample:** Isolated RNA from NP/OP Swab of suspected COVID-19 patients.

b) **Storage:** Specimen- At 4° C for 48 to 72 hours &

At -20°C for 10 days

Extracted RNA-After extraction the RNA was used for PCR test within 2 hours maintaining 4°C, in case of delay it was stored at -20^{0}C for up to 10 days. The freeze/thaw cycles could not exceed two.

3.12.3 Equipment, Materials, and Reagent

Equipment

a) Real-time PCR machine, Model- ABI QuantStudio 5 dx Real-Time PCR System by ThermoFisher Scientific

b) Minicentrifuge (Extragene, Model-EG600330)

c) PCR Cabinet (Model: PCR-100, Biobase, China)

d) Vortex (Thermofisher Scientific, Model: M16710-33)

e) -20°C Freeze- for reagent storage (Samsung, Model: RZ32M)

f) 2-8°C Refrigerator for reagent and chemical storage (AUCMA, MODEL:SC37)

g) CASIO MJ-120D plus Calculator

Materials

a) 1.5 ml Nuclease-free Micro-centrifuge tube

b) 100-1000 µL Pipettes (ratio pipette)

c) 20-200 µL Pipettes

d) 2-20 µL Pipettes

e) 0.2-2 µL Pipettes

f) Reaction Tube 8 strip- 0.1 ml

g) Reaction Cape 8 strip- 0.1 ml

h) 70% Ethanol

i) Rnase Free Filter tips 2-20 µl

j) Rnase Free Filter tips 200 µl

k) Rnase Free Filter tips 1000 µl

l) CD Marker

m) Powder-free sterile gloves

n) Facial Tissue

o) Towel tissue

p) Ice Gel Pack

q) Ball pen

r) 1.5 ml Microcentrifuge rack (8X12)

s) 0.1 ml PCR Reaction tube rack (8X12)

t) 0.1% NaClO

u) 1000 ml discarded beaker

v) PCR reaction rack

w) PCR template desigh sheet

x) Extracted RNA

Reagents

Table 2: Kit Composition of SARS-CoV-2 (COVID-19) RT-PCR Test

Name of Reagent (Manufacturer-SansureBiotech, Origin-China)	**Quantity(24Tests)**	**Ingredients**	**emperature**
2019-nCoV-PCR Mix	624 µl/tube x 1	Primers(4.62%), Probes(1.15%), dNTPs(3.85%), MgCl2(0.77%), Rnasin(0.48%), buffer(89.13%)	-20º C
2019-nCoV-Enzyme Mix	96 µl/tube x 1	zyme (62.5%), zyme (37.5%)	-20º C
2019-nCoV-PCR-Positive Control	500 µl/tube x 1	In vitro transcriptional RNA containing target genes (ORF1 ab,N gene) and Internal standard gene fragments (Rnase P)	-20º C
2019-nCoV-PCR-Negative Control	500 µl/tube x 1	Normal Saline	-20º C

***Storage Condition:** The kit was stored at -20±5^{0}c and avoided from light. Opened reagent freeze/thaw cycles should not exceed three.

3.12.4 Test Principle

By using Real-time fluorescence quantitative RT-PCR technology on the fluorescence quantitative PCR machine(Figure-5), this assay utilizes the novel corona virus (2019-nCoV) ORF 1ab and the specific conserved sequence of coding nucleocapsid protein N gene as the target regions which are designed for the conserved sequence of double-target genes, achieve isolation of sample RNA through fluorescent signal changes.

The PCR Real-Time PCR detection system uses positive internal control, which monitors the presences of PCR inhibitors in test specimens by detecting whether the internal control signal is normal, to avoid a false negative result.

Figure5: Picture of Real-Time Quantitative PCR. (Model-QuantStudio 5, by Applied biosystems,USA).

3.12.5 Procedures

a) 26µl of PCR Mix and 4µl of Enzyme Mix were added into the labeled PCR reaction tubes

b) 20µl of extracted RNA or 20µl of Positive and Negative Control into the labeled PCR tubes. The final reaction mix volume will be 50µl.It is necessary to keepallcomponents at +2 °C to +8 °C during the PCR preparation.

c) The tubes closed, centrifuged shortly, inserted into the device, and let amplify according to the following PCR profile.

d) PCR test channel-

Table 3: Target and Channel for SARS-CoV-2 (COVID-19) RT-PCR

Target	**Channel**
ORF-1 ab gene	FAM
N gene	ROX
Internal Control	CY5

e) Amplification profile

Table 4: Amplification profile of SARS-CoV-2 RT-PCR

Steps	Temperature (^{0}C)	Time	cycles
Hold	50	30mins	1
Hold	95	1mins	1
PCR	95	15 seconds	45
	60	30 seconds	
	25	10 seconds	

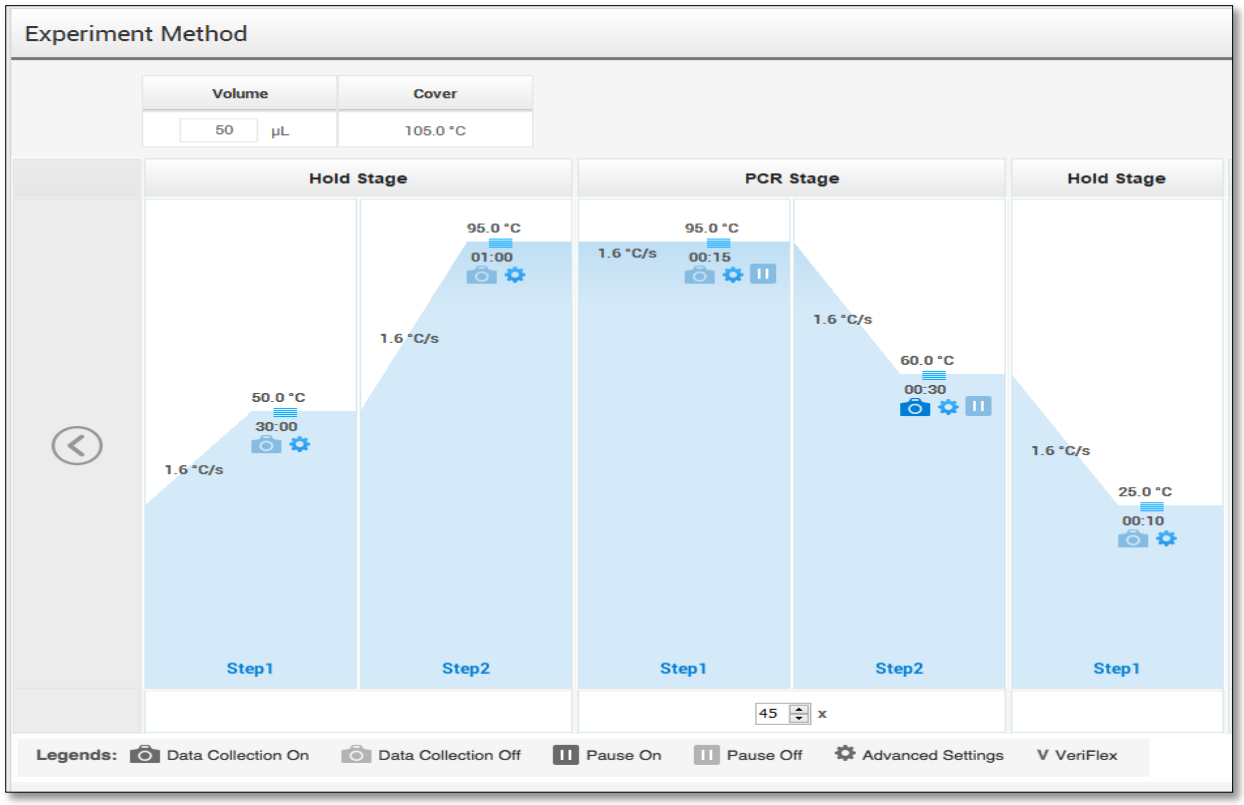

Figure6: Amplification Profile of SARS-CoV-2 RT-PCR

i. Quality Control

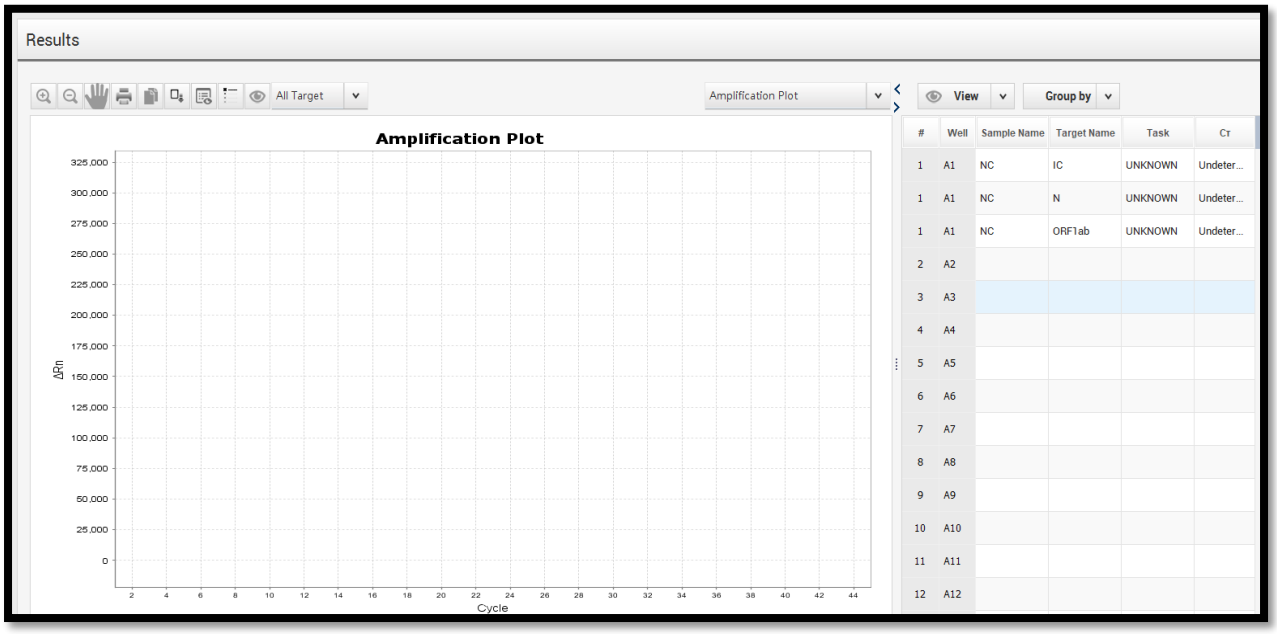

Figure 7: Amplification Profile of Negative Control(NC) of SARS-CoV-2 RT-PCR. In the figure, N-N gene(Undetected),IC-Internal Control (Undetected) and ORF1ab- Open Reading Frame1 ab gene (Undetected)

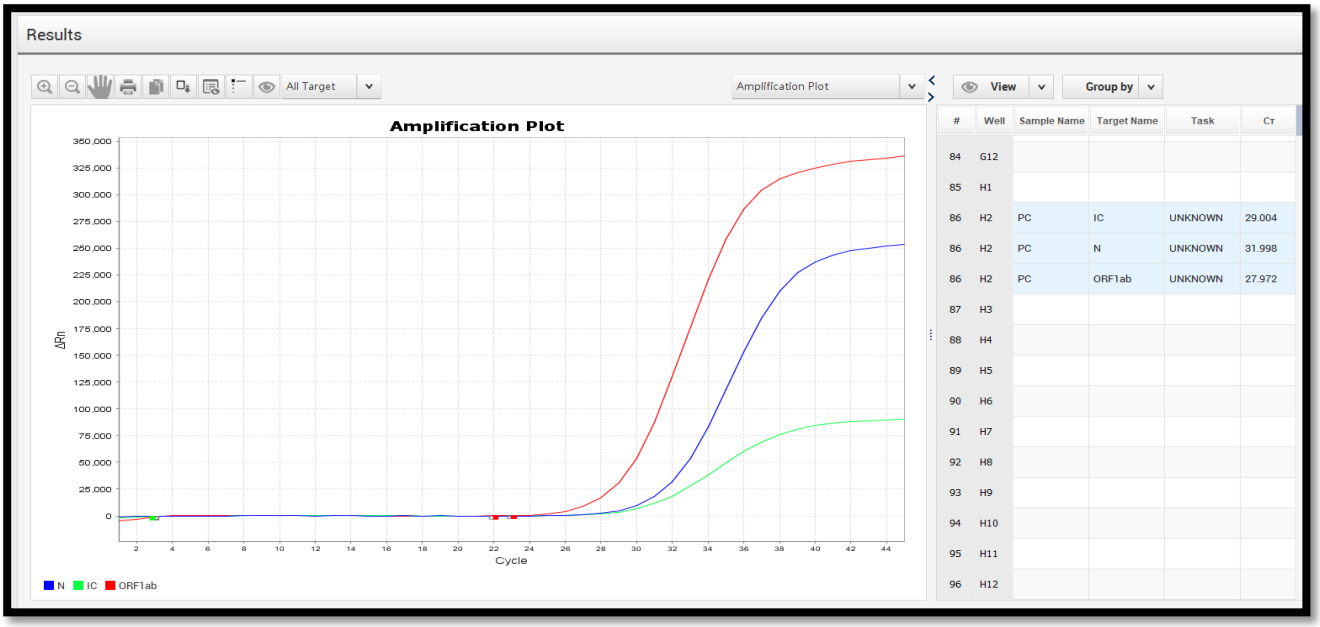

Figure 8: Amplification Profile of Positive control (PC) of SARS-CoV-2 RT-PCR. In the figure, N- N gene (Detected,CT-31.998),IC-Internal Control (Present,CT-29.004), and ORF1ab- Open Reading Frame1 ab gene (Detected,CT-27.972)

Table 5: Quality Control interpretation of COVID-19 RT-PCR

	2019-nCoV-PCR-Negative Control	2019-nCoV-PCR-Positive Control
Ct value	No Cycle of Threshold (Ct) or Ct > 40 at channel FAM,ROX and CY5 (internal control)	≤35 at channel FAM,ROX and CY5 (internal control)

ii. Results

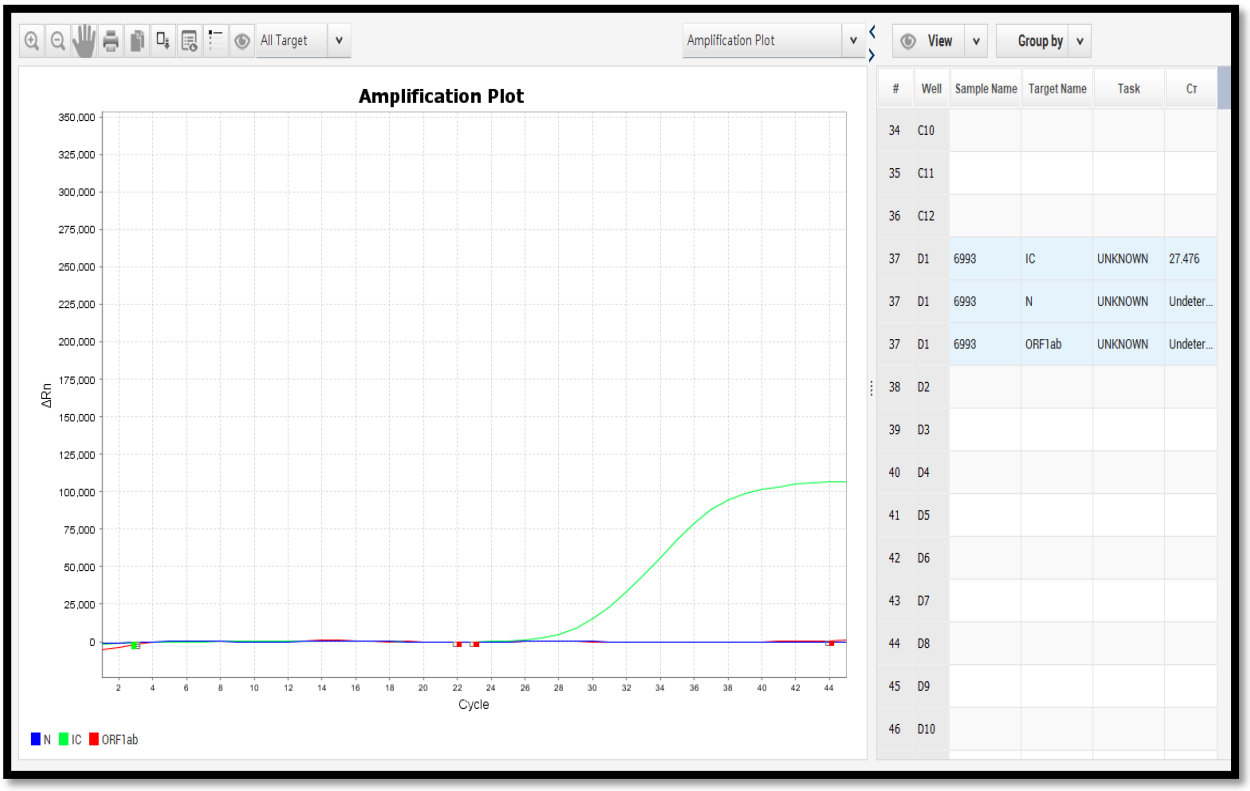

Figure 9: Negative amplification plot of SARS-CoV-2 RT-PCR. In the figure, N- N gene(Undetected),IC-Internal Control (Detected,CT-27.476) and ORF1ab- Open Reading Frame1 ab gene (Undetected).

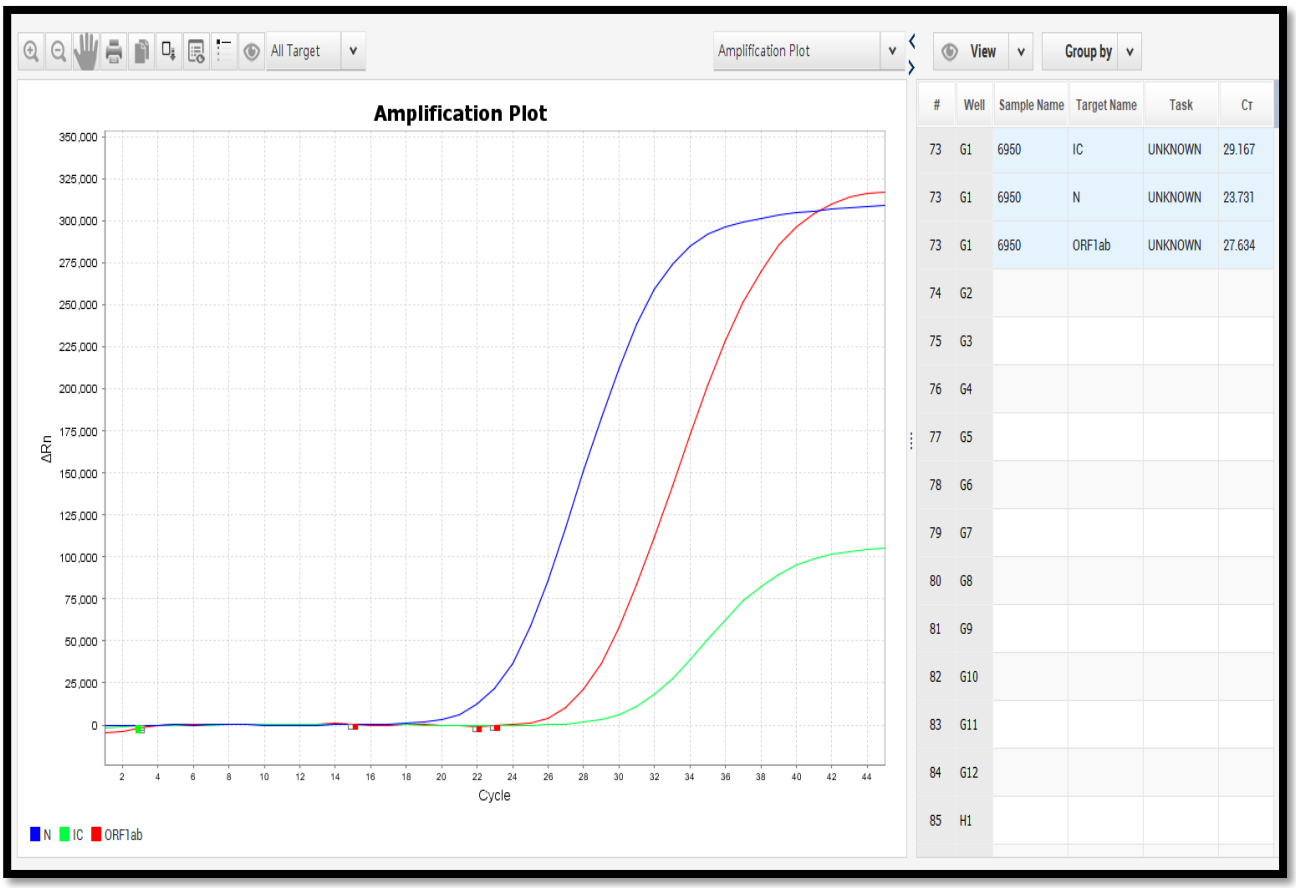

Figure 10: Positive amplification plot of SARS-CoV-2 RT-PCR (COVID-19) .In figure, N- N gene (Detected,CT-23.731),IC-Internal Control (Present,CT-29.167) and ORF1ab- Open Reading Frame1 ab gene (Detected,CT-27.637).

Table 6: Results interpreting criteria of SARS-CoV-2 Real-Time PCR

Conclusion	Amplification Result
2019-nCoV positive (Figure-7)	There was a typical S-shape amplification curve detected at FAM and/or ROX channel for ORF1ab & N gene Respectively. The Internal Contrl (IC) amplification curve which had detected at CY5 channel. Cycle of threshold (Ct) was ≤ 40.
2019-nCoV negative (Figure-6)	There is no typical S-shape amplification curve (No Ct) or Ct >40 detected at FAM and ROX channel, and For IC amplification curve which is detected at CY5 channel,Ct ≤ 40.

3.13 The procedure of Chest High Resolution of Computed Tomography (HRCT)

3.13.1 Test Principle

High-resolution CT (HRCT) of the chest, is a technique in which thin-slice chest images are obtained and post-processed in a high-spatial-frequency reconstruction algorithm. This test obtains images with exquisite lung detail, which are ideal for the assessment of diffuse interstitial lung disease. The most preferable chest HRCT protocol is expiratory images. The expiratory image protocol was followed in this study.

3.13.2 Test Methods

Expiratory HRCT imaging corresponds to an additional CT acquisition accomplished as part of the HRCT chest guideline. It represents a scan performed with the patient on supine and images acquired at the end-expiration.

It is a useful protocol for detecting small airway obstructive lung disease, in which the air is leftover trapped in the pulmonary lobules even after the expiration (air-trapping). This method may also be applied in the assessment for tracheobronchomalacia, although a dedicated method with a small ROI focused in the central airways is preferred.

3.13.3 Materials and Equipment

a. Patients gown
b. PPE (Apron, N95 Mask, Head Cover, Shoe Cover)
c. Hexisol
d. Patient’s identification
e. Dress changing room
f. Equipment- SEMENS SOMATOM High-Resolution CT Scan (Figure-11)

Equipment Specification

Table 7: Technical specification of HRCT scan

Characteristics	**Description**
Model	SEMENS SOMATOM definition flash High-Resolution CT Scan
Detector	2 x Stellar detector
Number of slices	2 x 128
Rotation time	0.28 s3
Temporal resolution	75 ms[3], heart-rate independent
Generator power	200 kW (2 x 100 kW)
kV steps	70, 80, 100, 120, 140 kV
Isotropic resolution	0.33 mm
Cross-plane resolution	0.30 mm
Max. scan speed	458 mm/s[3] with Flash Spiral
Table load	up to 307 kg / 676 lbs[3]
Gantry opening	78 cm

Figure 11: Schematic Diagram of SEMENS SOMATOM definition flash HRCT Scan machine

(Ref. - www.siemens-healthineers.com/vn/computed-tomography)

3.13.4 Chest HRCT Scan Procedure

Patient Preparation

a. The physician referred the patients to the CT scan department by the patients care attendant (PCA).

b. CT scan Medical Technologist checked the patient's identity.

c. The patient was told to remove any neck and Chest ornaments or metal.

Image Acquisition

a. Patients Position: The patient's position was supine with their arms above their head
b. Scout View: apices of tomogram was taken
c. Scan extent: apices of program
d. Scan Direction: The direction was Caudocranial
e. Contrast injection considerations: No contrast was needed
f. Scan delay: it was at a minimal label
g. Respiration phase: scan performed on expiration mode
h. Patient's directional points: The patient was taught, also practiced, how to perform and hold a full expiration within a few seconds. Time was taken between axial slices to give the patient a break

Post Processing

a. Firstly all of the pictures were taken in different modes by a CT-Scan technologist
b. Secondly, 100-150 good picture was selected and send to the consultant for reporting

Reporting

a. Findings: Lungs physiology, Ground Glass Opacities, Consolidation, Crazy paving, pleural effusion, lymphadenopathy, heart size, thoracic skeleton, and thoracic soft tissues condition were checked.
b. Ct Severity Score: The infection percentage of Lungs and severity score was analyzed
c. Conclusion: Diagnosis of Pneumonia, Bronchitis, Infection Stage, Lung infection percentage, and remarks was written in the report

3.14 The procedure of Laboratory Investigations

3.14.1 Biochemical Test

3.14.1.1 C Reactive Protein (CRP)

Methods Principle

C-reactive protein (CRP) has long been acknowledged as one of the most, if not the most, sensitive of the acute-phase reactants. C-reactive protein value in plasma can rise dramatically after myocardial infarction, stress, trauma, infection, inflammation, surgery, or neoplastic proliferation. The increment happens within 24 to 48 hours, and the level may be 2000 times normal. Because of the increase is nonspecific, however, it cannot be interpreted without a complete clinical history, and even then only by comparison with previous findings. Cord blood usually has low CRP concentrations (0.01 - 0.35 mg/L), but in intra-uterine infection, levels may be high as 260 mg/L.

For unknown reasons, the level of C-reactive protein response varies in some diseases that are otherwise apparently similar. For example, the C-reactive protein response in systemic lupus and ulcerative colitis, even when there are explicit signs and symptoms of

inflammation, is slight in contrast to its very large response in rheumatoid arthritis and Crohn's disease.

Methods

Immunoturbidimetric determination of C-reactive protein

Materials and Equipment

a. Apron
b. Gloves
c. Sample Cup
d. 1 ml autoclaved tips
e. 4 ml Vacutainer Rack
f. CD marker
g. 70% Ethanol
h. Discarded Beaker
i. 0.1% Sodium hypochlorite (NaOCl)
j. Sample-Red top Serum

Reagents

Beckman Coulter C - Reactive Protein (CRP)

REF-OSR6147

Reagent Storage and Stability

a. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C

b. Opened bottles of reagent are stable for 90 days when stored in the refrigerated compartment of the analyzer

Equipment

1. Thermo scientific Megafuge™ 8 Small Benchtop Centrifuge
2. Eppendorf 200-1000 Microliter Micropipette
3. Beckman Coulter AU680 Biochemistry Automated Analyzer

Procedure

1. Serum was separated from the sample vacutainer by centrifugation at 3000xg for 5 minutes
2. 300 µl (Minimum) separated serum was taken into a sample cup
3. Sample ID, and Patients Information was entered into the AU680 analyzer
4. Then the machine was run for 10 to 15 minutes.
5. Then the result was recorded
6. The result unit was mg/L

Reference Value

Less than 5.0 mg/L

3.14.1.2 D-dimer

Methods Principle

This method is a sandwich immunoluminometric assay. Use an anti-D-dimer monoclonal antibody to label ABEI, and use another monoclonal antibody to label Fluorescein-5-isothiocyanate (FITC). Sample, Calibrator or Control, ABEI Label, FITC Label, and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37°C, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time Subsequently,

the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is determined by a photomultiplier as Relative Luminescence Units (RLU) within 3 seconds and is proportional to the concentration of D-DIMER present in controls or samples.

Methods

Chemiluminescence immunoassay (CLIA)

Materials and Equipment's

a. Apron
b. Gloves
c. Sample Cup
d. 1 ml autoclaved tips
e. 4 ml Vacutainer Rack
f. CD marker
g. 70% Ethanol
h. Discarded Beaker
i. 0.1% Sodium hypochlorite (NaOCl)
j. Sample- Plasma with 3.2% Sodium citrate

Reagents

Maglumi PCT (CLIA)

REF-130216001M:100 tests

Storage and Stability

1. Sealed: stored at 2-8ºC until the expiration date.
2. Opened at 2-8ºC; minimum stability is 4 weeks.

3. On-board: Stable for 4 weeks.

4. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work.

5. Keep upright for storage to facilitate proper resuspension of microbeads.

6. Keep away from sunlight.

Equipment

a. Thermo scientific Megafuge™ 8 Small Benchtop Centrifuge

b. Eppendorf 200-1000 Microliter Micropipette

c. Snibe Diagnostic Maglumi 2000 Plus

Procedure

1. Plasma was separated from sample 3.2% Sodium citrate vacutainer by centrifugation at 3000xg for 5 minutes
2. 300-500µl separated plasma was taken into the sample cup
3. Sample ID, and Patients Information was entered into the Maglumi 2000+
4. Then the machine was run for 30 minutes.
5. Then the result was recorded
6. The result unit was µg FEU/mL

Reference Value

<0.50 µg FEU/mL

3.14.1.3 Procalcitonin (PCT)

Methods Principle

The PCT test is a sandwich chemiluminescence immunoassay. The sample (or calibrator/control, if applicable), buffer, ABEI labeled with anti-PCT monoclonal antibody, and magnetic microbeads

coated with another anti-PCT monoclonal antibody are mixed thoroughly and incubated, forming sandwich complexes. After precipitation in a magnetic field, decant the supernatant and then perform a wash cycle. Subsequently, the starter 1+2 is added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as a relative light unit (RLUs) which is proportional to the concentration of PCT present in the sample (or calibrator/control, if applicable).

Methods

Chemiluminescence immunoassay (CLIA)

Materials and Equipment

a. Apron
b. Gloves
c. Sample Cup
d. 1 ml autoclaved tips
e. 4 ml Vacutainer Rack
f. CD marker
g. 70% Ethanol
h. Discarded Beaker
i. 0.1% Sodium hypochlorite (NaOCl)
j. Sample- Red top serum

Reagents

Maglumi PCT (CLIA)

REF-130216001M: 100 tests

Storage and Stability

1. Sealed: stored at 2-8ºC until the expiration date

2. Opened at 2-8ºC; minimum stability is 4 weeks

3. On-board: Stable for 4 weeks

4. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work

5. Keep upright for storage to facilitate proper resuspension of microbeads

6. Keep away from sunlight

Equipment

a. Thermo scientific Megafuge™ 8 Small Benchtop Centrifuge
b. Eppendorf 200-1000 Microliter Micropipette
c. Snibe Diagnostic Maglumi 2000 Plus

Procedure

1. Serum was separated from the sample Red top vacutainer by centrifugation at 3000xg for 5 minutes
2. 40-300µl separated serum was taken into a sample cup
3. Sample ID, and Patients Information was entered into the Maglumi 2000+
4. Then the machine was run for 30 minutes.
5. Then the result was recorded
6. The result unit was ng/mL

Reference Value

<0.05 ng/mL

3.14.2 Immunological Test

3.14.2 .1 Interleukin 6 (IL-6)

Methods Principle

This method is a sandwich immunoluminometric assay. The sample (or calibrator/control,if applicable),buffer, ABEI labeled with anti-IL-6 monoclonal antibody and magnetic microbeads coated with another anti-IL-6 monoclonal antibody are mixed thoroughly and incubated, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the starters 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light unit (RLUs) which is proportional to the concentration of IL-6 present in the sample (or calibrator/control, if applicable).

Methods

Chemiluminescence immunoassay (CLIA)

Materials and Equipment

a. Apron
b. Gloves
c. Sample Cup
d. 1 ml autoclaved tips
e. 4 ml Vacutainer Rack
f. CD marker
g. 70% Ethanol
h. Discarded Beaker
i. 0.1% Sodium hypochlorite (NaOCl)
j. Sample- Red top serum

Reagents

Maglumi IL-6 (CLIA)

REF-130216004M:100 tests

Storage and Stability

1. Sealed: stored at 2-8ºC until the expiration date.

2. Opened at 2-8ºC; minimum stability is 6 weeks.

3. On-board: Stable for 4 weeks.

4. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work.

5. Keep upright for storage to facilitate proper resuspension of microbeads.

6. Keep away from sunlight.

Equipment

d. Thermoscientific Megafuge™ 8 Small Benchtop Centrifuge

e. Eppendorf 200-1000 Microliter Micropipette

f. Snibe Diagnostic Maglumi 2000 Plus

Procedure

7. Serum was separated from sample Red top vacutainer by centrifugation at 3000xg for 5 minutes
8. 100-300µl separated serum was taken into a sample cup
9. Sample ID, and Patients Information was entered into the Maglumi 2000+
10. Then the machine was run for 30 minutes.
11. Then the result was recorded
12. The result unit was pg/mL

Reference Value

<= 7.0 pg/mL

3.14.3 Hematological Test

3.14.3.1 WBC (White Blood Cells)-Total Count (TC)

Methods Principle

SF Cube is a pathbreaking technology for reliable blood cell count, including WBC, Differential, Reticulocytes, and NRBC with efficient flagging. After reaction with closed system reagents, the targeted blood cells undergo 3D analysis using information from the scatter of laser light at two angles and fluorescence signals. The total WBC count is primarily based on the Baso channel with an additional difference of WBC information from three other dedicated optical channels to eliminate the interference from NRBC, Lyse-resist RBC, etc.

Methods

SF Cube Cell Analysis Technology

Materials and Equipment

a. Apron
b. 4 ml Vacutainer Rack
c. CD marker
d. 70% Ethanol
e. Discarded Beaker
f. 0.1% Sodium hypochlorite (NaOCl)
g. Sample- Whole Blood With K3 EDTA

Reagents

a. Mindray-DS-DILUENT
b. Mindray-M-6FD DYE
c. Mindray-M-6FN DYE
d. Mindray-M-6LD LYSE
e. Mindray-M-6LH LYSE
f. Mindray-M-6LN LYSE
g. Mindary-Prove Cleanser

Storage

Stored at 18-25ºC until the expiration date

Equipment

a. Digisystem Roller Mixer,RM-500
b. Eppendorf 200-1000 Microliter Micropipette
c. Mindray 6200 Automated Hematology Analyzer

Procedure

a. The sample was rotated by a roller mixer at 40 rpm.
b. Sample ID, and Patients Information was entered into the Mindary 6200
c. Then sample vacutainer enter into the autoloader
d. The minimum sample requirements in the tube are 100-2000 µl
e. Then the result was recorded
f. The result unit was- For Total Leucocyte-x10^9/L and for Differential count-%

Reference Value

Total Leucocyte Count- 4.0-10.0 x 10^9/L

Neutrophils- 40-80 %

Lymphocyte-20-40 %

Monocytes- 2-10 %

Eosinophils-1-6 %

Basophils-1-2%

3.14.3.2 Platelet

Methods Principle

In the SF Cubec cell analysis technology, platelets (PLT) can be separated from the other interfering cell populations. Platelet-O results avoid interference from microcytic and fragmented RBCs, large platelets, and/or platelet clumps by fluorescent stain, and increase the accuracy and sensitivity of the results. PLT result is automatically corrected by the Focusing Flow-DC method when PLT-O counting mode is employed.

Methods

Focusing Flow-DC method and SF Cube Cell Analysis Technology

Materials and Equipment

a. Apron

b. 4 ml Vacutainer Rack

c. CD marker

d. 70% Ethanol

e. Discarded Beaker

f. 0.1% Sodium hypochlorite (NaOCl)

g. Sample- Whole Blood With K3 EDTA

Reagents

h. Mindray-DS-DILUENT

i. Mindray-M-6FD DYE

j. Mindray-M-6FN DYE

k. Mindray-M-6LD LYSE

l. Mindray-M-6LH LYSE

m. Mindray-M-6LN LYSE

n. Mindary-Prove Cleanser

Storage

Stored at 18-25ºC until the expiration date.

Equipment

d. Digisystem Roller Mixer, RM-500

e. Eppendorf 200-1000 Microliter Micropipette

f. Mindray 6200 Automated Hematology Analyzer

Procedure

a. The sample was rotated by a roller mixer at 40 rpm.

b. Sample ID, and Patients Information was entered into the Mindary 6200

c. Then sample vacutainer enter into the autoloader

d. The minimum sample requirements in the tube are 100-2000 µl

e. Then the result was recorded

f. The result unit was- For Total Platelet-x10^9/L

Reference Value

Total Platelet Count- 150-450 x 10^9/L

3.15 Statistical Analysis

A one-way Analysis of Variance (ANOVA) test was performed using the https://www.openepi.com/Mean/ANOVA website to compare the means of two or more group-independent variables. Sample size, Mean, and standard deviation of each parameter of the patient group were entered in ANOVA calculator for significance mean means difference. A p-value of less than 0.05 was considered significant. Results were illustrated by scientific graphical software namely GraphPad Prism 5. Chi-Square Calculator for 5 X 5 (or less) Contingency Table (sosscistatistics.com/test) was performed for find out the p value. The p-value of less than 0.05 was considered significant also.

Chapter 4: Results

4.1 Demographic Characteristics

In this study, a total of 135 COVID-19-affected patients were admitted from the different areas of chattogram at the year of June 2021 to July 2021. From these admitted patients we found that 62.7% (n=94) were male, while the rest 37.3% (n=56) were female. The mean age of the patients was 49 years. The COVID-19 diseases were detected by Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). All the patients were examined by the physician for clinical signs and symptoms to determine COVID-19. High-resolution computed tomography (HRCT) was performed in all enrolled patients.

4.2 Patients Group Based on Chest HRCT

In this study, high-resolution computed tomography (HRCT) was performed on all hospitalized patients. The CT scan images were analyzed according to the Fleischner Society Guideline, and the CT scan findings were categorized into three groups - 1) Normal HRCT, 2) Ground Glass Opacities (GGO) HRCT, and 3) GGO with Consolidation (GGO+Co) HRCT.

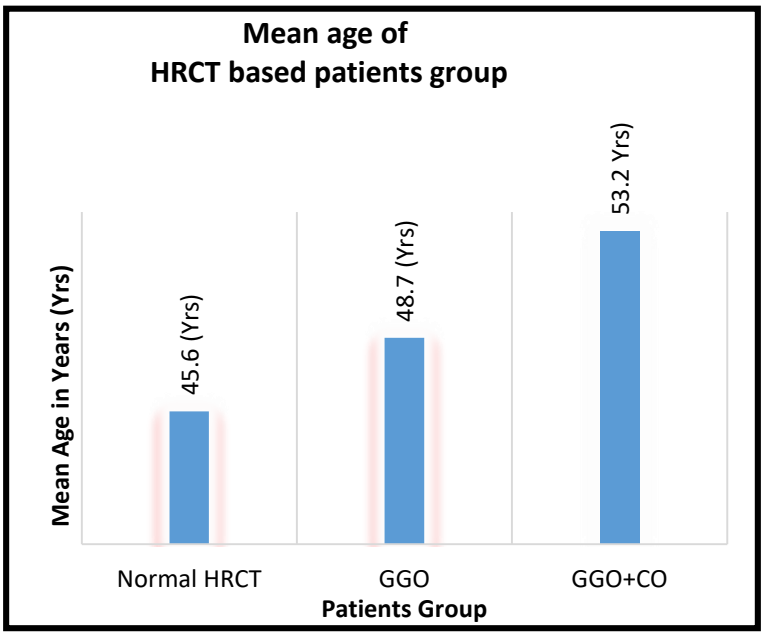

Figure 12: Mean age among three HRCT-based groups of COVID-19 enrolled patients.

The mean age of patients in the Normal, GGO, and GGO+Co groups were 45.6, 48.7, and 53.2 years, respectively (Figure-12). The Normal group had the lowest mean age (lower adults), while the middle-aged adults were identified in the GGO group and the highest mean age (highest adults) was found in the GGO+Co group
(https://www.statcan.gc.ca/en/concepts/definitions/age2).

4.2.1 Normal HRCT

Several important organs were analyzed in the chest CT, such as lung structure, nodules, bronchi, heart size, thoracic skeleton, and thoracic soft tissue. In the figure, the pulmonary structure was normal and showed normal vascular markings. The hilar region on each side was unremarkable, and the main bronchi appeared normal. The heart configuration and cardiac chambers were found to be of normal size. The thoracic skeleton and thoracic soft tissue showed no abnormalities (Figure-13_a+b).

4.2.2 Ground glass opacity (GGO)

In this group, bilateral multifocal diffuse ground-glass opacities with crazy paving were found. Usually, no pleural effusion or pneumothorax was seen. Hilar or mediastinal lymphadenopathy was not observed. The evaluation of hilar lymph nodes was limited without contrast. Heart size, thoracic skeleton, and thoracic tissue were found to be normal (Figure-13_c+d).

4.2.3 GGO+Consolidation (Co)

In this case, bilateral multifocal diffuse ground-glass opacities with consolidation in a peripheral distribution with scattered areas of intralobular lines ("crazy paving") and traction bronchiectasis were found. Usually, no pleural effusion or pneumothorax was seen.

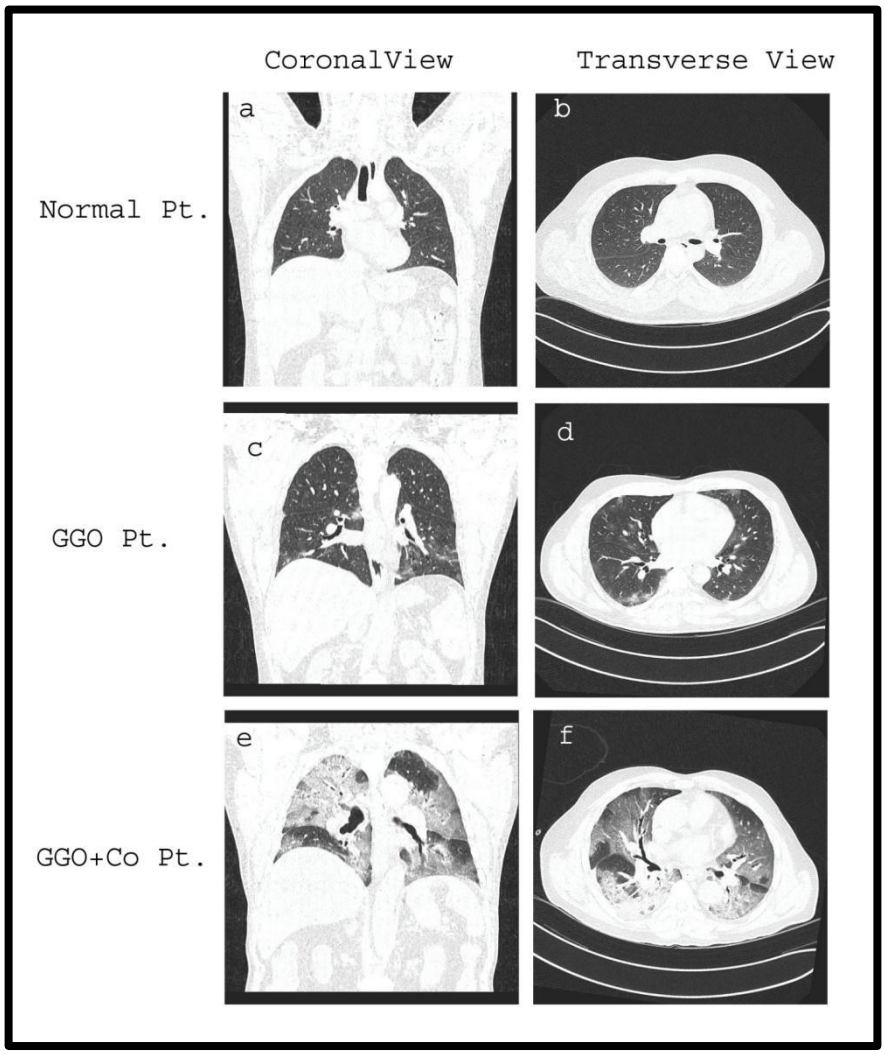

Figure 13: Show the image of chest HRCT in hospitalized COVID-19-detected patients. a+b- Coronal View and Transverse View of Normal Patients, b+c- Coronal View and Transverse View of GGO patients, and c+d Coronal View and Transverse View of GGO+Co patients. **(Ref. - www.siemens-healthineers.com/vn/computed-tomography/User manual)**

Heart size, thoracic skeleton, and thoracic tissue were found to be normal (Figure-mediastinal lymphadenopathy was not observed. The evaluation of hilar lymph nodes was limited 2_e+f).

4.3 Percentage of COVID-19 Detected Patients Based on HRCT

The chest HRCT was performed in 135 COVID-19-detected hospitalized patients, among them the normal HRCT (High-Resolution Computed Tomography) Group was found to be 36% (N=48).

Ground Glass Opacity (GGO) cases had been found to be 21% (N=29). The detection of Ground Glass Opacity with Consolidation (GGO+Co) group was high and the percentage showed 43% (N=58) (Figure-14).

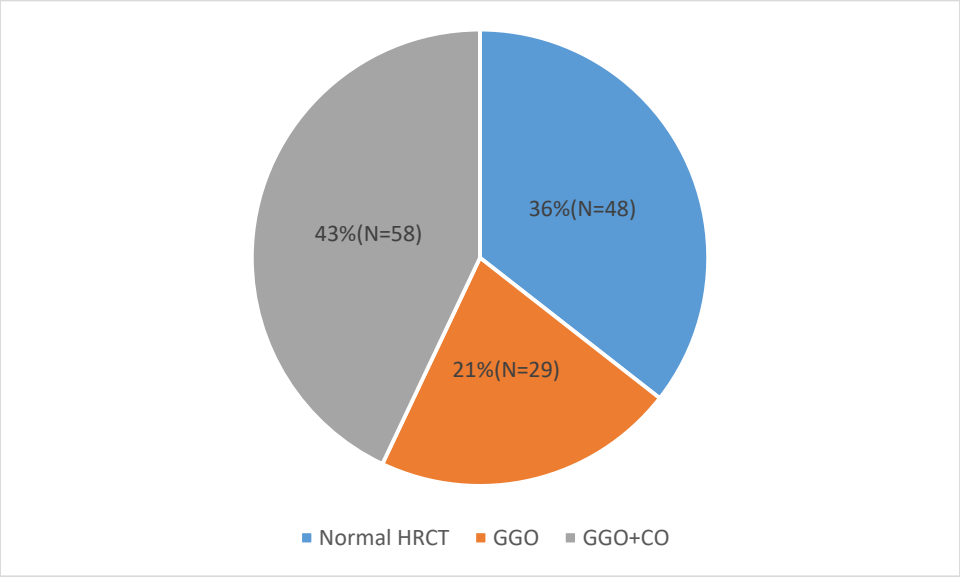

Figure 14: Differentiation of HRCT findings in covid-19 hospitalized patients. In the figure, HRCT= High-resolution computed tomography, GGO=Ground glass opacity, GGO+CO= Ground Glass Opacity with Consolidation.

4.4 Infection level analysis among different patients groups

Infection levels were calculated based on the infections found in the HRCT scans. All the patients with normal findings (N=48), those with ground-glass opacity (GGO) (N=29), and those with GGO with consolidation (N=58) were divided into five categories: normal (0-4%), mild (5-24%), moderate (25-49%), severe (50-74%), and critical (75-100%).

Figure-15 showed that there was no infection in the normal patient group. In the GGO group, mild (75.86%), moderate (13.79%), and severe (10.34%) cases were found, with mild cases being predominant. In contrast, in the patient group with GGO with consolidation, mild, moderate, and

severe cases were detected in 24.13%, 50%, and 24.13% of cases, respectively, with moderate-level infections being the most predominant. Critical cases were also found in 1.72% of cases (Figure-15).

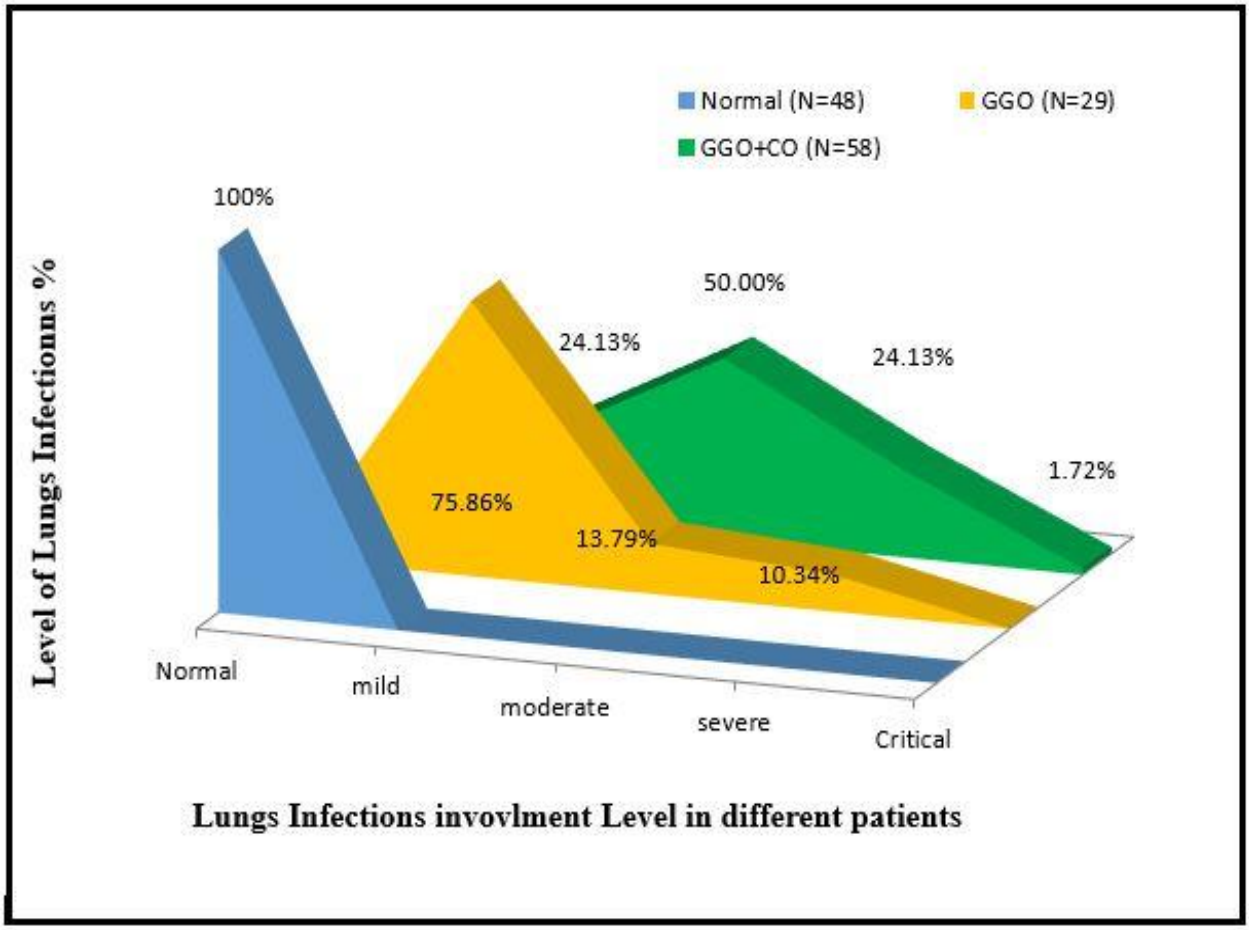

Figure 15: Shows the percentage of infection levels in different HRCT groups.

4.5 Clinical Manifestations Spectrum in HRCT- Tested Patients Groups

Clinical symptoms were recorded when patients came to the emergency COVID-19 unit in tertiary care hospitals in chattogram city. The most common symptoms of all patients (normal, GGO, and GGO+Co) were fever, cough, and headache where the fever was most predominant (96.55%, 92.85%, and 91.67% respectively) than cough in Normal (68.08%),GGO(71.42%) and GGO+Co(72.41%). Respiratory distress, sore throat, loss of smell and taste, and shortness of breath

were also common in all groups of patients but showed higher percentages in the GGO+Co group which were 43.10%, 32.75%, 29.31%, and 17.24% respectively.

Table 8: Clinical manifestation variable among hospitalized HRCT based patient group. Chi-Square Calculator for 5 X 5 (or less) Contingency Table (sosscistatistics.com/test) was performed among clinical manifestation variable of three groups. p=<0.05 was considered significant.NA-Not Applicable.

Variables	Normal (N=48)	GGO (N=29)	GGO+Co (N=58)	P Value
Clinical Manifestation				
Fever (%)	**44(91.67%)**	**26(92.85%)**	**56(96.55%)**	**0 .404**
Loss of smell and taste (%)	**9(19.14%)**	**5(17.85%)**	**17(29.31%)**	**0.310**
Headache (%)	**13(27.65%)**	**9(32.14%)**	**24(41.37%)**	**0.280**
Cough (%)	**32(68.08%)**	**20(71.42%)**	**42(72.41%)**	**0.811**
Shortness of Breath (%)	**2(4.25%)**	**4(14.28%)**	**10(17.24%**	**0.109**
Respiratory distress (%)	**4(8.51%)**	**6(21.42%)**	**25(43.10%)**	**0.00019***
Sore throat (%)	**4(8.51%)**	**7(25%)**	**19(32.75%)**	**0.010**
Vomiting (%)	**1(2.12%)**	**0(0.00%)**	**3(5.17%)**	**NA**
Loose Motion(%)	**4(8.51%)**	**1(3.57%)**	**8(13.79%)**	**0.0283**

Vomiting was not found in the patients' group having GGO without consolidation but was found in a tiny percentage in the normal patients group and patients having GGO with consolidation (2.12% and

5.17% respectively) (Table-8)

4.6 Condition-Based Oxygen Saturation (SpO_2) in HRCT Patients Groups

Based on oxygen saturation (SpO_2) during COVID-19 infection three different criteria mild (≥95% SpO_2 on room air), moderate (90-94%SpO_2), and severe (<90%SpO_2) conditions of patients determined among three different groups [Normal (N=48), GGO (N=29), and GGO+Co(N=58). The figure illustrates that there is a linear relationship betweenthe oxygen saturation of infected patients with a different group of patients based on HRCT scans (Normal, GGO, and GGO+Co). In this study, in the Normal patient group, Mild, Moderate,and Severe SpO_2 levelsshowed [N=47,(97.92%)],[N=1(2.08%)[, and [N=0,(0%)] respectively. SpO_2 levels, Mild [N=27, (93.10%)], Moderate [N=2,(6.89%)],and Severe [N=0,(0%)] were detected in GGO patient groups.

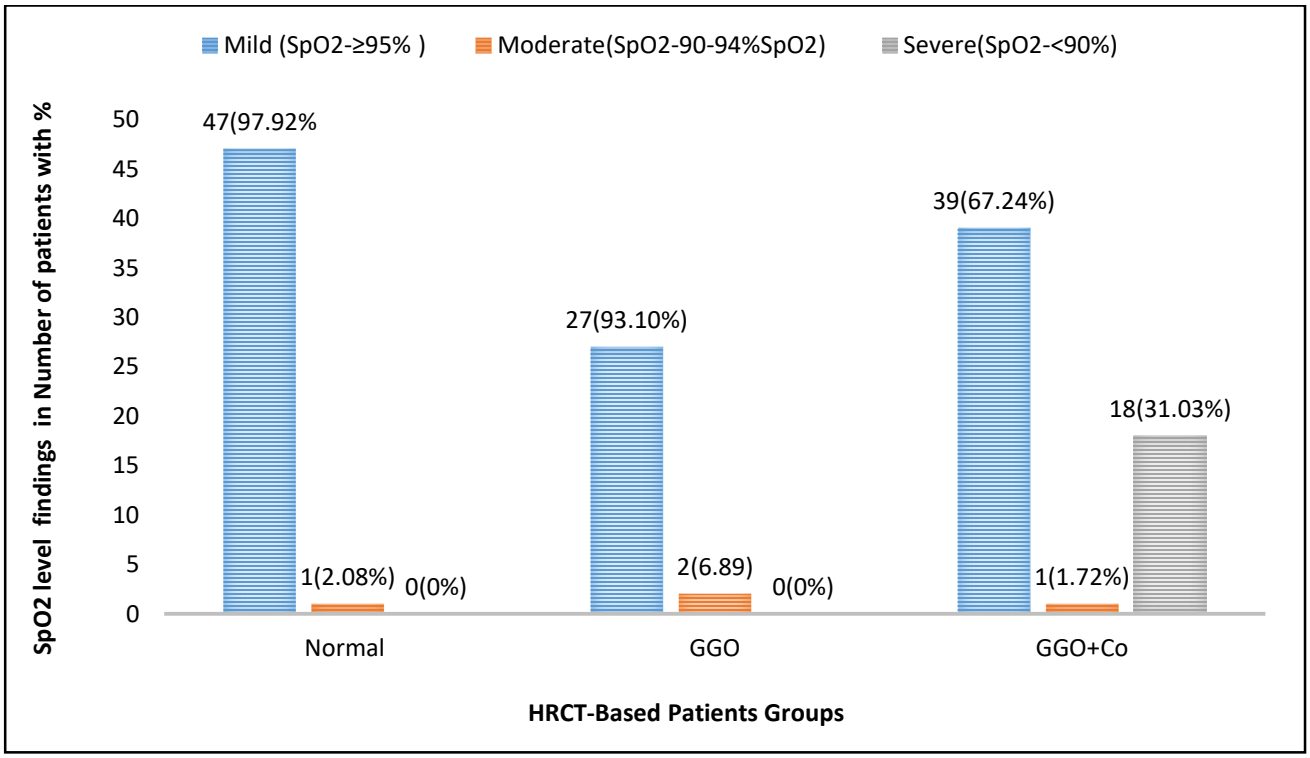

Figure 16: Oxygen saturation of different groups of patients (Mild≥95%, Moderate 90-94%, Severe <90%).

No severe cases were observed in GGO and GGO+Co. On the other hand, SpO_2 level [N=39, (67.24%)] = Mild, [N=1,(1.72%)] = Moderate and only [N=18,(31.03%)=Severe cases were found in GGO+Co patients groups

Linear regression showed that oxygen saturation has a linear relationship with the infection of patients found in HRCT scans (Normal < GGO <GGO+Co). In severe cases showed a positive relationship with the infection increased (Figure-17).

4.7 Relation of Biochemical Marker among HRCT patients

4.7.1 Comparison of CRP Levels among HRCT-Tested Patients

The C-reactive proteins (CRP) were analyzed in the Normal, GGO, and GGO+Co groups. The normal reference value for CRP is <5.0 mg/L, as stated in the user manual of Beckman Coulter Inc.

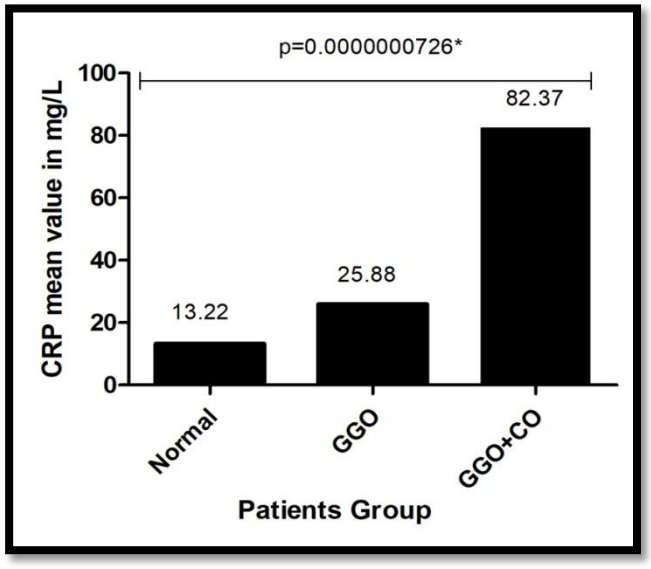

Figure 17: Comparisons of CRP Mean in COVID-19 detected HRCT-based patients group. In the Figure, HRCT- High-resolution computed tomography, GGO- Ground glass opacity, GGO+CO (Ground glass opacity with Consolidation), $\bar{x}$= Mean. OpenEpi, Version 3, open source calculator-

ANOVA test was performed between three groups and a significant p-value (p=0.000000077*) had found p= (p=<0.05 was considered significant).

(Ref-OSR6147_EN_C-Reactive Protein_ User manual_ Beckman Coulter Inc., USA). As shown in Figure-18, the mean CRP of the Normal group was 13.21 mg/L. The mean CRP value in the GGO group was found to be higher, at 35.70 mg/L, compared to the Normal group. The highest mean CRP value, 82.37 mg/L, was detected in the GGO+Co group, in contrast to the Normal and GGO groups. A significant p-value (p=0.000000077*) was found among the three groups (Figure-18).

4.7.2 Comparisons of D-Dimer Levels among Chest-CT Scan Patients Groups

D-dimer is an important biomarker for COVID-19-detected patients and this test was performed in all cases.

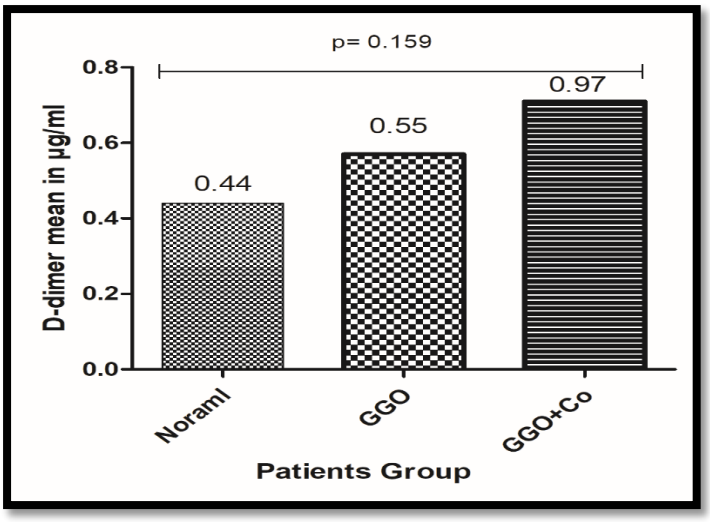

Figure 18: Differentiation of D-Dimer mean value in Normal, GGO, and GGO+CO groups in COVID-19 detected patients. OpenEpi, Version 3, open source calculator-ANOVA test was performed between three groups and p-value (p=0.159) had found p= (p=<0.05 was considered significant).

The normal value of D-dimer is <0.5 µg/ml (Ref-130206008M-D-dimer-V1.0-EN-20110716_MAGLUMI_CHINA). The mean of D-dimer in the Normal group was 0.44 µg/ml.On the other hand, D-dimer (0.55 µg/ml) found to be in GGO patient group. The maximum D-dimer value (0.97 µg/ml)was had been detected in GGO+Co groups. Statistically, a p-value was found (p=0.159) among the three groups of COVID-19-detected enrolled patients (Figure-18).

4.7.3 Comparisons of (PCT) Levels in HRCT-Based COVID-19 Patients Groups

Procalcitonin was tested in all COVID-19 admitted patients, with a reference value of <0.05 ng/mL (Ref-6p22-27_PCT_Architech_USA). The mean Procalcitonin (PCT) value in the Normal group was 0.14 ng/mL. In GGO cases, the PCT value was higher at 0.22 ng/mL compared to the Normal patient group. The highest PCT value of 0.68 ng/mL was found in the GGO+Co group.

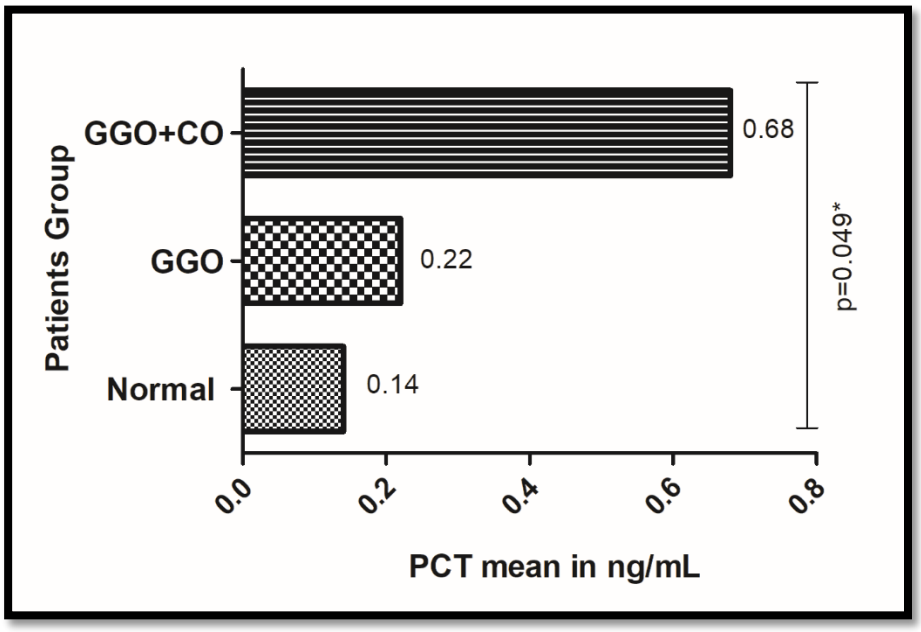

Figure 19: Comparison of the mean value of PCT among the HRCT-diagnosed case. OpenEpi, Version 3, open source calculator-ANOVA test was performed between three groups, and a significant p-value (p=0.049*).

There was a significantdifference in PCT level (p=0.049) among the Normal, GGO, and GGO+CO patient groups. PCT values showed a linear relationship with the infection of patients found in HRCT scans (Normal < GGO < GGO+CO)(Figure-19).

4.8 Relation of Hematological Profile among HRCT Patient Groups

4.8.1 Age and gender of Hematological profile tested in COVID-19 detected patients

Table 9: Hematological profile tested in COVID-19-detected patients among different age and gender groups

Pt. Group	<60		≥60	
	Male	Female	Male	Female
Normal (48)	23 (47.92%)	12 (25%)	9 (18.75%)	4 (8.33%)
GGO (29)	19 (65.52%)	6 (20.69%)	1 (3.44%)	3 (10.34%)
GGO+CO (58)	24 (41.38%)	9(15.52%)	15 (25.86%)	10 (17.24%)

Age and gender were analyzed in the Normal, GGO, and GGO+Co patients groups for hematological findings. Two age categories were used: less than 60 and 60 years or older. In the Normal group, 47.92% were male and 25% were female in the <60 age category, 18.75% were male and 8.33% were female in the ≥60 age category. In the GGO group, 65.52% were male and 20.69% were female in the <60 age category, 3.44% were male and 10.34% were female in the ≥60 age category. In the GGO+Co group, 41.38% were male and 15.52% were female in the <60 age category, 25.86% were male and 17.24% were female in the ≥60 age category (Table9).

4.8.2 Hematological Analysis of Normal Patient Group

Table 10: Hematological parameters with CRP in Normal Patient Group. Two sample-independent t-tests was performed & for each group $p<0.05$ was considered significant (openepi.com/Mean/t test)

Hematology Parameter with CRP (Normal)	**Ref.** Value	**Unit**	**< 60 age**		**60 ≥ age**		**P value between (< 60& ≥60 age)**
			Male-Mean	**Female-Mean**	**Male-Mean**	**Female-Mean**	
CRP	<5.0	mg/L	17.41	9.80	7.90	13.95	0.001722*
TC	4-10	10^9/L	6.575	5.446	11.482	6.196	0.05068
Eosinophil	1-6	%	1.12	2.308	2.45	2.5	0.1348
Neutrophil	40-80	%	56.78	65.1	62.93	51.425	0.1318
Lymphocyte	20-40	%	25.45	23.65	29.95	35.48	0.2977
Platelet	150-450	10^9/L	201.35	253.33	199.75	211.5	0.5971

Hematological Parameters CRP, TC, Eosinophil, Neutrophil, Lymphocyte, and Platelet count were measured and found very significant data of CRP ($p=0.001$) but TC, Eosinophil, Neutrophil, Lymphocyte, and Platelet count was not significantly different from the reference value in normal HRCT patients ($p=0.05$, $p=0.13$, $p=0.13$, $p=0.29$, $p=0.59$). Both males and female of the age of below or above 60 have had these hematological parameters in their normal ranges (Table-10)

4.8.3 Hematological Findings of Patients with Ground Glass Opacity (GGO)

Table 11: Hematological parameters with CRP in GGO Patient Group. Two samples independent t-test was performed & for each group $p<0.05$ was considered significant (openepi.com/Mean/t test)

Hematological Parameters with CRP(GGO)	**Ref.** Value	**Unit**	**< 60 age**		**60 ≥ age**		**P value between (< 60& ≥60 age)**
			Male	**Female**	**Male**	**Female**	
CRP	<5.0	mg/L	17.62	19.26	155	17.62	0.0001505*
TC	4-10	10^9/L	8.29	5.678	8.35	8.29	<0.0000001*
Eosinophil	1-6	%	1.39	0.72	0	1.39	0.07666
Neutrophil	40-80	%	67.17	50.34	77.9	67.17	0.3215
Lymphocyte	20-40	%	23.16	23.38	16	23.16	0.4202
Platelet	150-450	10^9/L	249.6	178	174	249.6	0.09063

Hematological Parameters CRP, TC, Eosinophil, Neutrophil, Lymphocyte, and Platelet count were measured and found very significant data for CRP and TC (p=0.001, p=<0.0000001) but Eosinophil, Neutrophil, Lymphocyte, and Platelet count did not differ significantly in HRCT patients having GGO (p=0.07, p=0.32, p=0.42, p=0.09). Both males and females of the age below or above 60 have had these hematological parameters in their normal ranges except males of 60 or above ages have a low number of eosinophil counts than the normal range and CRP is higher than other age criteria (Table 11).

4.8.4 Hematological Outcomes of Patients with GGO + Consolidation (Co)

Table 12: Hematological parameters with CRP in GGO+Co Patient Group. Two sample independent t test was performed & for each group p<0.05 was considered significant, Ref-openepi.com/Mean/t-test)

Hematology Parameter with CRP (GGO+Co)	**Ref.** Value	**Unit**	**< 60 age**		**≥ 60 age**		**P value between (< 60& ≥60 age)**
			Male-Mean	**Female-Mean**	**Male-Mean**	**Female-Mean**	
CRP	<5.0	mg/L	53.50	85.67	133.98	60.48	0.2166
TC	4-10	10^9/L	6.60	4.71	7.89	7.29	0.3756
Eosinophil	1-6	%	0.3	0.41	1.04	0.1	<0.0000001*
Neutrophil	40-80	%	69.54	67.42	77.24	78.07	0.5471
Lymphocyte	20-40	%	22.23	25.76	16.61	19.39	0.7440
Platelet	150-450	10^9/L	221.74	199.57	227.4	304.71	0.02065

Hematological Parameters CRP, TC, Eosinophil, Neutrophil, Lymphocyte, and Platelet count was measured and found very significant data in the case of eosinophil count (p=<0.0000001) but CRP, TC, Neutrophil, Lymphocyte, and Platelet count did not differ significantly in HRCT patients having GGO+Co (p=0.21, p=0.37, p=0.54, p=0.74, p=0.02). Both male and female of age of below or above 60 have had these hematological parameters in their normal ranges except Eosinophil of both male and female of below 60 age and female of 60 or above 60 age have a low number of eosinophil count than normal range. CRP elevation found in male of above 60 ages (Table-12).

4.8.5 Comparison of Hematological Features Among Patient Groups in Logarithmic Values

The bar diagram showed the comparisons of hematological features (CRP, TC, Eosinophil, Neutrophil, Lymphocyte, and Platelet count) of different patient groups (Normal, GGO, GGO+Co) and showed that Neutrophil, and Platelet count (72.45%, 231.92 10^9/L) were highest in GGO+Co group than GGO and normal group but eosinophil and Lymphocyte count had an inverse increasing direction where in normal it was in highest count number (1.89%, 27.81%) than GGO and GGO+Co. However, In the case of TC, the GGO group has the highest count number (7.56 10^9/L) than the normal group and GGO+Co (Figure-20).

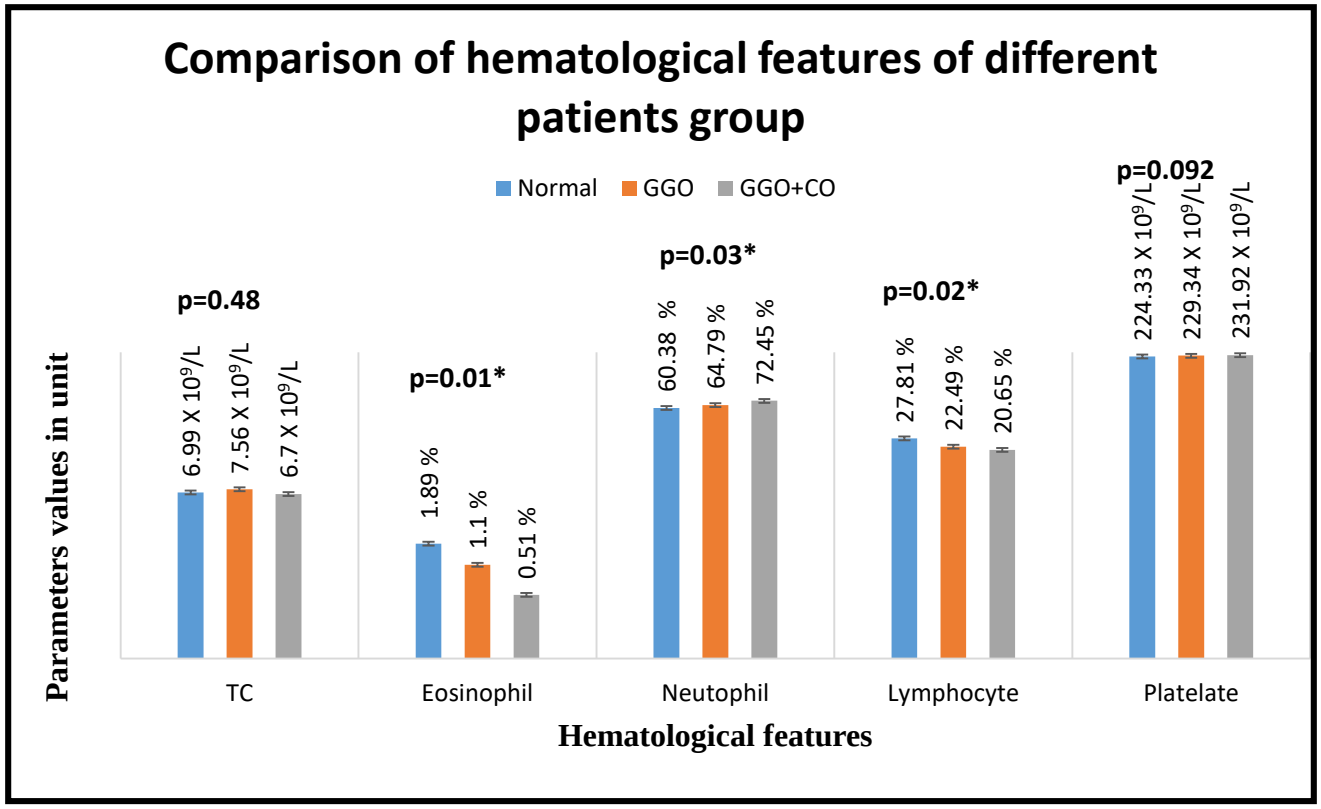

Figure 20. Comparison of hematological features of different HRCT-based patients group in logarithmic value. (Refernece Value of TC= 4-10 X 10^9/L,Eosinophil=1-6%,Neutrophil=40-80%, Lymphocyte=20-40 % & Platelet=150-450 10^9/L)

4.9 Relation of Interleukin-(IL-6) Among HRCT-Tested Patient Groups

Interleukin-6 (IL-6) is a significant prognostic immunological biomarker in critically ill COVID-19 hospitalized patients. The normal reference value for IL-6 is <=7.0 mg/L, as stated in the user manual of Maglumi, Snibe Diagnostics (Ref-152 IL-6-en-EU, V1.0, 2018-08_ user manual of Maglumi, Snibe Diagnostics _ China). IL-6 levels were analyzed in COVID-19-enrolled patients.

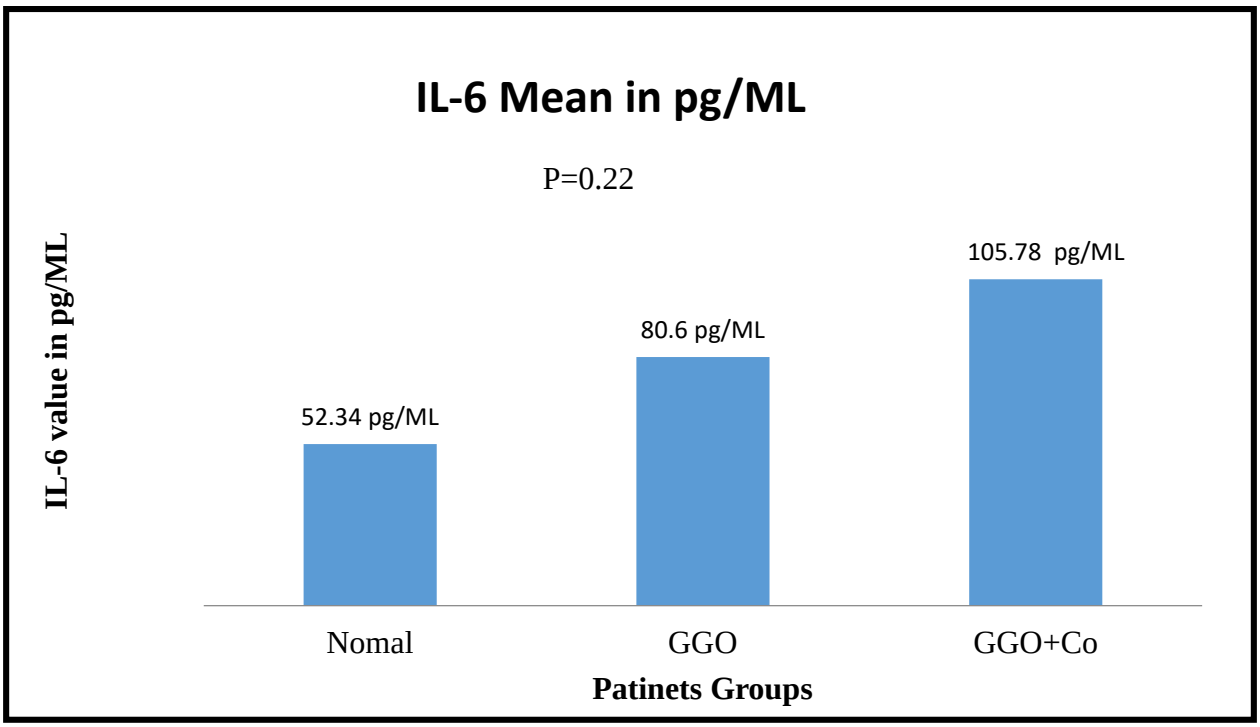

Figure 21. The IL-6 findings in COVID-19 were compared among the normal, GGO, and GGO+Co patient groups.

The Patients with the worst condition were found to have higher values of IL-6. The highest IL-6 mean values were found in the GGO+Co patients group at 105.78 pg/ML and Normal & GGO patients groups were found to be 52.34 pg/ML & 80.6 pg/ML. (Figure-21).

Chapter 5: Discussion

Chest imaging is a part of the diagnosis of COVID-19 disease when RT-PCR testing is not available, in case of delayed test results, or when there is a clinical suspicion of COVID-19 with initial negative or inconclusive RT-PCR results (Use of Chest Imaging in COVID-19: A Rapid Advice Guide, 11 June 2020, 2020). A CT scan can be a useful tool in evaluating the individual disease burden. The most common CT findings in COVID-19 patients are ground-glass opacities, consolidation and interlobular septal thickening. These findings are associated with increased levels of inflammatory markers such as CRP, Procalcitonin and interleukin-6 as well as decreased levels of lymphocytes (Li et al., 2020, Huang et al., 2020 & Lessmann et al., 2020).

In this study, the clinical symptoms manifested by 135 COVID-19 patients along with their Imaging and laboratory parameters of COVID-19 patients, particularly hematological, immunological and biochemical parameters were observed. The study aimed to find out the relation of chest CT findings with the parameters and set a biomarkers for the treatment of COVID-19 patients.

There were 62.7% male patients, while the rest 37.3% were female and the mean age of the patients was 49 years. Our study findings were supported with one study in Bangladesh which found 62.78% and 37.22% male & female patients respectively and their mean age was 49.55 (Bulbul *et al.,* 2021).

Based on HRCT density, the patients were classified into 3 groups i.e. Normal (36%), GGO (21%), and GGO+Co (43%). The Infection levels were calculated based on lung involvement and categorized into Normal, mild, moderate, severe, and critical groups. Our study showed that there was no infection in the normal group, but in the GGO and GGO+Co groups infections were observed. Mild (75.86%), moderate (13.79%), and severe (10.34%) cases were found in the GGO and there were 24.13%, 50%, and 24.13% infections in the mild, moderate, and severe cases in the

GGO+Go Group, respectively. A critical case (1.72%) was found in the GGO+Co group only. Similar data was also found in Abu Dhabi, Europe, and England where a large number of the patient required oxygen for their treatment having severe CT scan report and found a statistically significant correlation of CT severity with oxygen requirements (Saeed et al., 2020, Europea. Eu., 2020, Ackermann et al., 2020)

The clinical manifestations shown by COVID-19 patients included fever (88.82%), cough (37.65%), sore throat (15.29%), mylgia (48.82%), headache (42.94%), diarrhea (1.76%,), lack of taste and smell (46.47%) (Bulbul et al., 2021). A 2020 study in China found fever (88.7%) and cough (67.8%) as the most common and diarrhea (3.8%) as less common(Guan et al., 2020 & Wang et al., 2020). In this study, we observed the same clinical symptoms manifested by 3 groups of COVID-19 patients where the fever was most predominant in normal (91.67%), GGO (92.85%), and GGO+Co (96.55%) than cough in Normal (68.08%), GGO(71.42%) and GGO+Co(72.41%).

Another study showed fever as the most predominant in all groups of CT patients having 90.9%, 94.7%, and 95.5% fever in normal, GGO, and consolidation group (Fraghaly et al., 2022). In this research, respiratory distress, sore throat, loss of smell and taste, and shortness of breath were also common in all groups of patients. There were higher percentages, namely 43.10%, 32.75%, 29.31%, and 17.24% of the mild, moderate, and severe cases, respectively in the GGO+Co groups. Another study showed that the common symptoms were respiratory problems, cough, and chest pain (Fraghaly et al., 2022).

Oxygen saturation (SpO2) is a crucial parameter for monitoring the condition of COVID-19 patients. In this study, SpO2 levels were measured in three groups of COVID-19 patients: namely,Normal, GGO, and GGO+Co. In the Normal group, mild cases accounted for the highest proportion (97.92%), moderate cases accounted for 2.08%, and there were no severe cases. In the GGO group, no severe

cases were observed, and the proportions of mild and moderate cases were 93.10% and 6.89%, respectively. Among the patients with GGO with consolidation, mild and moderate cases accounted for 67.24% and 1.72%, respectively, while severe cases accounted for 31.03% of the group of three. Our study is consistent with a previous study, which found that the proportion of mild cases with respect to SpO2 levels was 17.1%, while moderate cases accounted for 82.9% in the hospitalized COVID-19 patients (Qadir et al., 2022).

A comparison of clinical and laboratory parameters was made. Among the laboratory markers, GGO+Co group had higher levels of CRP (82.37mg/L), D-dimer (0.97µg/ml), PCT (0.68ng/mL) than Normal cases who had 13.22 mg/L(CRP), 0.44 µg/ml (d-Dimer and 0.14 ng/mL(PCT) and GGO cases had CRP (25.88mg/L), D-dimer (0.55) and PCT (0.22ng/mL) and the comparison generated a significance ($p<0.05$) for CRP and PCT. These findings for CRP, D-dimer, and ferritin in the patients with consolidation with another published data, where CRP value was 3.3, 21.8, and 70 and D-dimer was 0.3, 0.5, and 0.6 in the normal, GGO, and GGO+Co groups, respectively (Farghaly et al., 2022).

However, hematological parameters i.e. total Count, eosinophil, neutrophil, lymphocyte, and platelet count were measured for 3 groups of patients below or above 60 ages. The findings concluded that all the parameters were in the normal range in the normal group but the patients above 60 ages had higher CRP, neutrophil, and platelet count but lower number of eosinophil and lymphocyte counts in the GGO and GGO+Co groups.

Raised platelet count, CRP, D-dimer, and procalcitonin level had been reported by several studies in COVID-19 patients (Huang et al., 2020, Bulbul et al., 2021). Another domain that is thought to predict the severity of COVID-19 is the neutrophil:lymphocyte (N:L) ratio; a higher N:L ratio is postulated to be related to more severe outcomes.

Moreover, immunological parameters i.e. Interleukin-6 was also measured for 3 groups of patients and it was found that the GGO+Co group had the highest IL-6 levels than GGO and normal groups. The Patients with the worst condition were found to have higher values of IL-6 and this finding is consistent with the study which postulated that IL6 was associated directly with the severity of coronavirus disease-2019 (COVID-19) (Talwar et el., 2022).

Our result showed that HRCT findings had a significant correlation with CRP level, Procalcitonin, D-dimer level, oxygen requirements, and other hematological and immunological features. Studies also suggested CRP as a predictive marker for the likelihood of disease progression (Infectious Disease Advisor,2020). D-dimer likewise can be used as a prognostic indicator. However, It is not yet clear whether this increase is related to the direct effect of the virus or the systemic inflammatory response [Yao et al., 2020, Graniliski et al., 2015]. Procalcitonin levels usually elevate mildly in viral infection, whereas significant elevation is seen in fungal, bacterial, or parasitic infections [Zhao et al., 2022]. Our study was capable of confirming the D-dimer, CRP, and procalcitonin to be higher in the GGO+Co patients' group, implying that the inflammatory response was noticeably more evident in the patients with a severe form of COVID-19.

Conclusion

This study concludes that chest CT scans can play a pivotal role in assisting physicians in the management plan and work as an indicator of disease severity and possible outcome. The CT infection percentage is positively correlated with the inflammatory laboratory markers and oxygen requirement in the patients with COVID-19 infection. In the case of suspected false-negative SARS-CoV-2 RT-PCR results, a chest HRCT scan can be used as an alternative method for the diagnosis and treatment of COVID-19 disease. RT-PCR testing is recommended as the gold standard method for the detection of COVID-19. However when RT-PCR is not available or results provide an initial negative or false positive or inconclusive data; analysis of clinical, biochemical, immunological, and hematological parameters can be used as a prognostic alternative for the treatment of patients, which can reduce the mortality rate. However, more research is needed to further clarify the evaluation of chest CT for prognosis of COVID-19 disease, including its correlation with patient outcome.

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Appendices

1. Instruments list

SL	Steps of the study	Instruments and tools	Model/Lot	Manufacturer	Country of Origin
1	Sample Collection	GELWELL COVID-19 Sample Collection Booth	884574	GETWELL PRAN-RFL	Bangladesh
2		Disposable sampling swab,Type-A-01	22012603	biocomma	China
		Lab Refrigerator (2-8^{o}C)	SC37	AUCMA	China
3	Sample Transportation	Marina Cooler Box (Sample Transportation Box)	6S	Lion Star Plastics	Indonesia
4		TCPplus Gel Packs	-	STORO Pack	Germany
5		ZipLock Bag 10''X14"	-	PRAN-RFL	BD
6	SARS-CoV-2 RNA Extraction	Biosafety Cabinet Class II	VBH 36C2	Angetantoni Lifescience (ALS)	Italy
7		Microcentrifuge, Refrigerated	Micro CL 17R	Thermo Fisher Scientific	USA
10		Mixer,Vortex	M16710-33	Thermo Scientific	USA
11		Single channel micropipette 2-20 µl	4780020	Ratio Lab	Germany
12		Single channel micropipette 20-200 µl	4780200	Ratio Lab	Germany
13		Single channel micropipette 100-1000 µl	4781000	Ratio Lab	Germany
14		Laboratory Freezer Solid Door	LSFSF39DC-UK	Lec MEDICAL	UK
16	SARS-CoV-2 RNA Real Time PCR (Qualitative)	PCR Cabinet	PCR-1000	BioBase	China
17		Single channel micropipette 2-20 µl	4780020	Ratio Lab	Germany
18		Single channel micropipette 20-200 µl	4780200	Ratio Lab	Germany
19		Single channel micropipette 100-1000µl	4781000	Ratio Lab	Germany
20		Mini Centrifuge	FC5306	OHAUS	USA
21		Mini Centrifuge	EG6000330	Extragene	Taiwan

22	SARS-CoV-2 RNA Real Time PCR (Qualitative)	PCR Rack, 0.1 mL, 0.2 m	0030124545	eppendrof	Germany
23		Real Time PCR	QuantStudio 5	applied biosystems	USA
24		Real Time PCR	ABI 7500 fast dx	applied biosystems	USA
25		Freeze (-18 ºC to -20 ºC)	RZ32M7120B1	Samsung	Vetnam
26	Chest HRCT Scan	SOMATOM definition flash HRCT Scan machine	SOMATOM definition flash High Resolution CT Scan	SEMENS	Germany
27	Statistical Analysis	GrapgPad prisom	Prisom 5	GraphPad Software	USA
28		Open epi	https://www.openepi.com/	Open epi programs	Emory University,USA
29	Biochemical Analysis	Biochemistry Auto Analyzer	AU680	Beckman Coulter	USA
30	Immunological Analysis	Immunology AutoAnalyzer	Maglumi 2000 Plus	Snibe Diagnostic	China
31	Hematological Analysis	Hematological AutoAnalyzer	Mindray 6200	Mindray	China

2. Sample Storage Reagent Composition

Reagent Name	Manufecturer	Specfication & Quantity			Main Ingredients
Sample Storage	Sansure Biotech	24 Test	48 Test	96 Test	Normal Saline,Rnasin and etc.
		2.0 mL x 24 tube	2.0 mL x 48 tube	2.0 mL x 96 tube	

3. Sample Release Reagent Composition

Reagent Name	Manufecturer	Specification & Quantity			Main Ingredients
Sample Release	Sansure Biotech	24 Test	48 Test	96 Test	Lysis buffer (SO3)
		1.2 mL x 1 tube	1.2 mL x 2 tube	L x 1 tube	

4. 70% Ethanol (CH_3CH_2OH)Preparation

Materials- a) 100% ACS grade CH_3CH_2OH

b) Phase II Deionized Water

Working Concentration-1000mL

Preparation- for 1000ML of 70% CH_3CH_2OH

Name of the Chemicals/Materials	Volume
100% ACS grade CH_3CH_2OH	700 mL
Phase II Deionized Water	300 mL
Total Amount	1000 mL

5. 1% Sodium Hypochlorite Preparation

Materials- a) 5 % Sodium Hypochlorite (NaOCl)

b) Phase II Deionized Water

Working Concentration-1%

Preparation- for 1000ML of 1% NaOCl

Name of the Chemicals/Materials	Volume
5 % Sodium Hypochlorite (NaOCl)	200 mL
Phase II Deionized Water	800 mL
Total Amount	1000 mL

6. Difference of CPR results among HRCT tested Group:

Patients (Pt.)Group	Number (N)	Mean ($\bar{x}$)	Std. Deviation (σ)
Normal	48	13.23	17.81
GGO	29	25.51	33.81
GGO+CO	58	77.25	83.04

Table 1. Number (N), Mean ($\bar{x}$) and Std. Deviation (σ) of CRP in Normal HRCT, GGO & GGO+CO diagnosed pt. group.

Source of variation	**Sum of squares**	**d.f**	**Mean square**	***F* statistics**	**p-value**
Between Groups	119430	2	59715.1	17.9159	0.000000130675*
Within Groups	439967	132	3333.08		
Total	559397	134			
Test for equality of variance	**Chi square** 97.9822	**d.f** 2	**p-value** 0.0000000000539248*		

Table 2. Open Epi ANOVA table of CRP. F and Chi square test was performed between patients groups for p value. P<0.05 was considered significant.

7. Comparisons of D-Dimer in Chest CT patients:

Pt. Group	Number (N)	Mean ($\bar{x}$)	Std. Deviation (σ)
Normal	48	0.44	0.36
GGO	29	0.57	0.55
GGO+CO	58	0.71	0.97

Table 3. Number, Mean and Std. Deviation of D-Dimer in HRCT tested patients.

Source of variation	**Sum of squares**	**d.f**	**Mean square**	***F* statistics**	**p-value**
Between Groups	1.92182	2	0.96091	1.86003	0.159725
Within Groups	68.1925	132	0.51661		
Total	70.1143	134			
Test for equality of variance	**Chi square** 45.2992	**d.f** 2	**p-value** 0.000000000201411*		

Table 4. Open Epi ANOVA table of D-Dimer. F and Chi square test was performed between patients groups for p value. P<0.05 was considered significant.

8. Number, Mean and Std. Deviation of PCT in HRCT diagnosed patients

Pt. Group	Number (N)	Mean ($\bar{x}$)	Std. Deviation (σ)
Normal	48	0.14	0.26
GGO	29	0.22	0.53
GGO+CO	58	0.68	1.76

Table5. Number, Mean and Std. Deviation of PCT in HRCT diagnosed patient

Source of variation	**Sum of squares**	**d.f**	**Mean square**	***F* statistics**	**p-value**
Between Groups	8.71581	2	4.35791	3.06624	0.0499294*
Within Groups	187.606	132	1.42125		
Total	196.321	134			
Test for equality of variance	**Chi square** 142.545	**d.f** 2	**p-value** 0.0000000000818772*		

Table.6 Open Epi ANOVA table of PCT. F and Chi square test was performed between patients groups for p value. P<0.05 was considered significant.

9. Normal Chest HRCT scan report sample

Clinical Info: COVID-19 RT-PCR Positive

Comparison: No relevant prior study available.

Technique : HRCT of the Chest.

Coronal and sagittal reformatted images are also obtained.

Findings:

Pulmonary structure is normal and shows normal vascular markings.

There are no intrapulmonary nodules or patchy opacities.

The hilar region on each side is unremarkable, and the main bronchi appearnormal.

The heart is normal in configuration. The cardiac chambers are of normal size.

The thoracic skeleton and thoracic soft tissues show no abnormalities.

Impression:

Normal Chest HRCT.

10. GGO chest HRCT scan report sample

CLINICAL INFO: COVID-19 RT-PCR Positive

TECHNIQUE: HRCT of the Chest.

Coronal and sagittal reformatted images are also obtained.

FINDINGS:

Bilateral multifocal diffuse ground-glass opacities with dilated vessels in a peripheral distribution with scattered areas of intralobular lines ("crazy paving") are noted.

No pleural effusion or pneumothorax are seen.

No hilar or mediastinal lymphadenopathy. Evaluation of hilar lymph nodes is limited without contrast.

Normal heart size. No pericardial effusion.

The thoracic skeleton and thoracic soft tissues show no abnormalities.

CT SEVERITY SCORE:

Affected Lung in Percentage Per Lobe - Max. 25 points

0% = 0 ; <5% = 1 ; 5-25% = 2 ; 25-50%= 3 ; 50-75% = 4 ; 75-100%= 5

RUL: 1, RML: 1, RLL: 2, LUL: 1, LLL: 2.

Total CT Severity Score: 7. The percentage of lung involvement is 28%

CONCLUSION :

CO-RADS 6: Known COVID-19 (PCR proven) Pneumonia, Progressive stage.

Total CT Severity Score: 7 [< 8 = mild]. The percentage of lung involvement is 28%

11. GGO+Co chest HRCT scan report sample

CLINICAL INFO: COVID-19 RT-PCR Positive

Technique : HRCT of the Chest.

Coronal and sagittal reformatted images are also obtained.

FINDINGS: Bilateral multifocal diffuse ground-glass opacities with consolidation in a peripheral distribution with scattered areas of intralobular lines (“crazy paving”) and traction bronchiectasis are noted.

No pleural effusion or pneumothorax are seen.

No hilar or mediastinal lymphadenopathy. Evaluation of hilar lymph nodes is limited without contrast.

Normal heart size. No pericardial effusion.

The thoracic skeleton and thoracic soft tissues show no abnormalities.

CT SEVERITY SCORE:

Affected Lung in Percentage Per Lobe - Max. 25 points

0% = 0 ; <5% = 1 ; 5-25% = 2 ; 25-50%= 3 ; 50-75% = 4 ; 75-100%= 5

RUL: 5, RML: 3, RLL: 4, LUL: 4, LLL: 5.

Total CT Severity Score: 21. The percentage of lung involvement is 84**%**

CONCLUSION:

CO-RADS 5: (COVID-19) Pneumonia (abnormalities highly suggestive for COVID-19), Peak stage.

Total CT Severity Score: 21 [>15 = Severe] The percentage of lung involvement is 84%

12. SARS-CoV-2(COVID-19) RT-PCR Sample Collection Form

COVID-19 SAMPLE COLLECTION & EXAMINATION (RT-PCR) FORM

Specimen ID*:		Date specimen collected*:	_____/_____/2022

সেবা গ্রহীতা পূরণ করবেন

নাম (ইংরেজি বড় হাতের অক্ষরে)/ NAME (in Capital Letter) *:		পাসপোর্ট নং/ Passport No*:					
মোবাইল নং/ Mobile No*:		মোবাইল মালিকের সাথে সম্পর্ক/ Relation with phone owner*:	নিজ Self	স্বামী/স্ত্রী spouse	সন্তান child	অন্যান্য other
বর্তমান ঠিকানা (বাসা/ রোড/ মহল্লা/ গ্রাম)/ Current Address (House/ Road/ Colony/ Village)*:		ইউনিয়ন/ ওয়ার্ড নং (সিটি)/ Union/ Ward No (City):				
উপজেলা/ থানা/ Upazila/ P.S*:		জেলা/সিটি কর্পোরেশন/ District/ City Corporation*:				
নমুনা সংগ্রহ কেন্দ্র/ Specimen Collection Centre*:						

●●● **বিশেষ দ্রষ্টব্য:** বিদেশগামী যাত্রীরা নমুনা প্রদানের পর থেকে যাত্রার সময় পর্যন্ত সম্পূর্ণ আইসোলেশনে থাকবেন/
Foreign travelers should maintain complete isolation after giving sample until the flight.
●●● Download your report before your flight from this link: covid19reports.dghs.gov.bd

নমুনা সংগ্রহকারীর স্বাক্ষর

IMPERIAL HOSPITAL LIMITED

COVID-19 SAMPLE COLLECTION & EXAMINATION (RT-PCR) FORM

Profile:

সেবা গ্রহীতা ও তার পরিচয় পূরণ করবেন

নাম (ইংরেজি বড় হাতের অক্ষরে)/ NAME (in Capital Letter) *:			পাসপোর্ট নং/ Passport No*:				
Nagad Transaction ID*:			ফ্লাইট নং/ Flight No*:				
মোবাইল নং/ Mobile No*:			মোবাইল মালিকের সাথে সম্পর্ক/ Relation with phone owner*:	নিজ Self	স্বামী/স্ত্রী spouse	সন্তান child	অন্যান্য other
জন্ম তারিখ (যদি জানা থাকে)/ Date of Birth:	DD-MM-YYYY		বয়স (১বছরের নিচে হলে "০" লিখুন)/ Age (Under 1 year, write "0")*:				
লিঙ্গ/ Gender*:	☐ পুরুষ/ Male ☐ মহিলা/ Female ☐ অন্যান্য/ Other		গ্রুপ ব্লাড Blood Group:				
বর্তমান ঠিকানা (বাসা/ রোড/ মহল্লা/ গ্রাম)/ Current Address (House/ Road/ Colony/ Village)*:			ইউনিয়ন/ ওয়ার্ড নং (সিটি)/ Union/ Ward No (City):				
			জেলা/সিটি কর্পোরেশন/ District/ City Corporation*:				
উপজেলা/ থানা/ Upazila/ P.S*:		ই-মেইল এড্রেস/ Email Address:					

নমুনা সংগ্রহকারী ও স্বাস্থ্যকর্মী পূরণ করবেন

Specimen Collection Centre:			
Facility contact person name:		Contact mobile no.:	

Clinical Assessment:

Any signs or symptoms:	☐ Fever	☐ Cough	☐ Shortness of Breath	☐ Sore throat	
Date of symptom onset: _____/_____/2022					

Specimen Collection & Lab Request:

Specimen ID*:		Date specimen collected*:	_____/_____/2022

Sample Classification*:	☐ New	☐ Follow up	☐ Contact	☐ Dead body	
Specimen details:	☐ Nasal Swab	☐ Throat Swab	☐ Serum		
Referred laboratory name*:					

Specimen collected by

ল্যাব কর্তৃক পূরণীয়

Lab Result and Notification:

Date of specimen received at lab*:	_____/_____/2022	Date of Lab Test Result*:	_____/_____/2022

Test Result*:	Negative	Positive	Inconclusive	Invalid	Not Performed
Corona test laboratory name*:					

তারকা (*) চিহ্নিত ঘরগুলো অবশ্যই পূরণ করতে হবে/Star(*) marked field must be filled up.

Examined by