RESEARCH ARTICLE

Estimation of emerging diagnostic parameters for Coronavirus Disease 2019 patients severity and fatality

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Abstract

Objective: To identify various emerging diagnostics parameters of coronavirus disease 2019 related to disease progression and fatality.

Method: The cross-sectional study was conducted at Mardan Medical Complex, Khyber Pakhtunkhwah, Pakistan, from February 9, 2021, to April 21, 2021, and comprised patients of either gender aged >18 years diagnosed with coronavirus disease 2019 on the basis of polymerase chain reaction testing and who were admitted to the hospital using the World Health Organisation interim guidelines. Disease progression was categorised as mild, moderate, severe and critical, and they were monitored closely till the final outcome. Data was analysed using SPSS 26.

Result: Of the 408 patients, 215(52.69%) were male and 193(47.30%) were female. The overall median age of the sample was 55 years (interquartile range: 18-84 years). Symptoms included cough 92(22.54%), fever 80(19.60%), shortness of breath 78(19.60%), fatigue 60(14.70%) and loss of smell and test 52(12.74%), while 46(11.27%) were asymptomatic. Azithromycin was the most used drug 304(74.50%), while antiviral Remdesivir was given to 279(68.38%) patients and hydrocortisone to 143(35.04%). Plasma treatment was given to 55(13.48%) patients and mechanical ventilation to 87(21.32%). Compared to baseline, disease progression was mild in 72(17.64%) patients, moderate 96(23.52%), severe 98(24.01%) and critical in 89(21.81%), while no change was seen 53(12.99%) cases. Severity level was significantly associated with liver and renal function parameters ($p<0.05$). Overall, 47(11.51%) patients died.

Conclusions: Different severity levels during hospitalisation among patients of coronavirus disease 2019 were noted, and severity level was significantly associated with liver and renal function parameters.

Keywords: Coronavirus disease 2019, Diagnostic tests, Progression, Fatality. (JPMA 72: 1384; 2022)

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Introduction

An outbreak of the novel coronavirus disease 2019 (COVID 19) was recorded in Wuhan, China, in December 2019. Since the pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS CoV 2) infection remains unclear, there is no standard therapy, and the majority of patients are treated symptomatically. Better knowledge of the disease's pathogenesis is important. Cases can often be misdiagnosed owing to clinical manifestations.[1]

C-reactive protein (CRP) and pro-inflammatory cytokines levels of interleukin-6 (IL-6) have been shown to be elevated in COVID-19 patients along with lower lymphocyte counts, but studies have also shown that patients with acute disease have increased cytokines levels, like IL-2, tumour necrosis factor-alpha (TNF- α) and IL-10, in plasma.[2]

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The gold standard for evaluating SARS-CoV-2 aetiology is nucleic acid analysis. Besides, the growing demand for real time reverse transcriptase polymerase chain reaction (rRT-PCR) assay testing has highlighted the shortcomings of such testing on a large scale, like its lengthy processing period.[3] Furthermore, rRT-PCR contains general analytical and preanalytical problems that can jeopardise the test's diagnostic accuracy.[4] Nonetheless, some recent studies have shown that the test can produce up to 20% false-negative (FN) outcomes.[5] As a result, there is an immediate need for alternative testing to rapidly classify SARS-CoV-2 patients to prevent viral transmission and to ensure prompt treatment.[6] Several haematological tests have been specifically affected in COVID-19 patients, and a blood test can help in the detection of false-positive (FP) or FN rRT-PCR which can also play an important role in large-scale screening of suspected COVID19 populations.[7]

The current study was planned to identify various emerging diagnostics parameters of COVID-19 related to disease progression and fatality.

Patients and Methods

The cross-sectional study was conducted at Mardan Medical Complex (MMC), Khyber Pakhtunkhwah, Pakistan,

from February 9, 2021, to April 21, 2021. MMC is the only referral hospital for COVID-19 patients in the city. After approval from the institutional ethics review committee, the sample was raised using convenience sampling technique from among patients of either gender aged >18 years diagnosed with COVID-19 on the basis of PCR testing and who were admitted using the World Health Organisation (WHO) interim guidelines.[8] The patients were enrolled for the study within the first 24 hours of admission after taking written informed consent.

Those excluded were patients having co-morbidities, those who were negative on rRT-PCR testing, those referred to another medical facility during hospitalisation, patients hospitalised more than once, and patients whose laboratory data was found missing.

Pharyngeal swabs for COVID-19 PCR testing were taken from each participant. To confirm SARS-CoV-2 in a pharyngeal swab sample using a QIAGEN Rotor-Gene Q rRT-PCR system and a Biomérieux ARGENE SARS-COV-2 R-GENE package licensed by the Food and Drug Administration (FDA) were used. The SARS-CoV-2 R-GENE kit includes two PCR assays. The first stage in the identification of SARS-CoV-2 is PCR1, while PCR2 is optional and can be conducted with the same mixture to test specimen consistency by verifying the existence of human cells and identify a third strongly preserved specific gene to Sarbeco virus. This real time PCR is purely based on the amplification of different regions of specific target genome. For detection, the 5' nuclease hydrolysis probe technique is used. The SARS-CoV-2 genome, in particular, was used in a triple reaction, with the N gene of SARS-CoV-2 at 530nm, the RdRp gene of SARS-CoV-2 at 670nm, and internal control at 560nm. The primers used in the PCR method allow the SARS-CoV-2 N gene to be amplified. The amplified fragment has a length of 148bp. The SARS-CoV-2 RdRp gene amplified fragment is 136bp long. Each study was conducted in triplicate; one sample, one positive and negative control each. These analytical standards were developed in accordance with FDA guidelines.[9]

Blood samples were collected for testing white blood cell (WBC) count, lymphocyte (LYM) count, LYM morphology, and platelets (PLT). Blood biochemistry parameters, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, albumin urea, creatinine, as well as creatinine phosphokinase (CPK), ferritin, D-dimer, CRP and lactate dehydrogenase (LDH), were measured using Sysmex XP-300 haematology analyser, an architect c400 clinical chemistry analyser and a Roche Cobas E411 automated immunoassay analyser.

Data from the institutional electronic database was obtained. Clinical and pathology laboratory data was collected on the first day of admission during the first clinical consultation. Treatment data and patient outcomes, including disease progression, duration of each disease stage, fatality, length of hospitalisation, and endpoint status, were also collected throughout the course of the study. Disease progression was defined as normal and abnormal at admission; normal meaning diagnostic parameter within the normal range, while abnormal meant the parameters exceeded the upper normal range, which would progress to severe or critical stage. Those without symptoms were initially classified as asymptomatic. Those having symptoms were categorised as mild, moderate, severe or critical.[2] The severity was defined in line with relevant guidelines.[10] Patients were classified as "mild" when there was no suggestion of imaging pneumonia or any moderate or higher/severe features; as "moderate" when evidence of imaging pneumonia existed, but without severe or higher/severe features; or as "severe" when meeting any of the following criteria: respiratory distress equivalent to 30 breathes per minute, oxygen saturation equivalent to 93% at room air, arterial oxygen partial pressure (PaO_2) or inspired oxygen fraction (FiO_2) of ~300mmHg equivalent to 0.133kPa). The patients were categorised as “critical” if they required mechanical ventilating, with septic shock and/or required admission to intensive care unit (ICU).[11]

Data was analysed using SPSS 26. Data was expressed as median and interquartile range (IQR) or as frequencies and percentages, as appropriate. Association between categorical variables, like gender, age, excluded comorbidities, symptoms and chest radiography, was explored using Pearson chi-square test or Fisher exact test. Inter-group differences were tested for severity of disease on the basis of asymptomatic, mild, moderate, severe and critical variables through one-way analysis of variance (ANOVA) Kruskal Wallis. Olympus Ep50 electronic

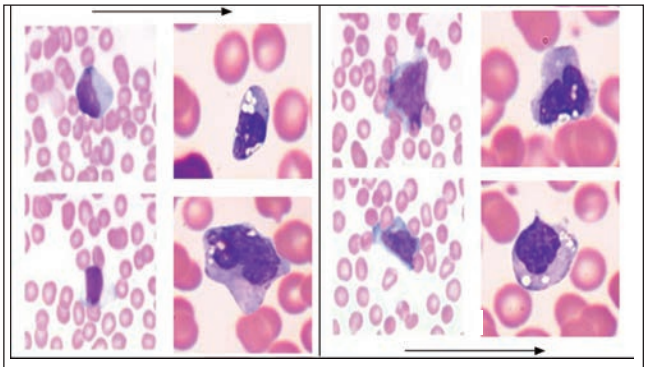

Figure-1: Lymphocyte morphology showing reactive (left) and apoptotic (right) lymphocytes.

microscope camera was utilised to analyse the Grünwald Giemsa stained smear for lymphocyte morphology images (800x600 pixels). All findings were compared across severity groups. Each patient provided a single sample. Each patient had 50-100 lymphocytes assessed in at least 20 randomly selected microscopic fields. A scoring system, based on GrünwaldGiemsa stain (Figure 1), was developed to help in the evaluation of LYM morphology.[12] Statistical significance was determined with $p<0.05$.

Results

Of the 805 cases examined, 408 were included; 215(52.69%) were male and 193(47.30%) were female. The overall median age of the sample was 55 years (IQR: 18-84 years). Symptoms included cough 92(22.54%), fever 80(19.60%), shortness of breath 78(19.60%), fatigue 60(14.70%) and loss of smell and test 52(12.74%), while 46(11.27%) were asymptomatic. Compared to baseline, disease progression was mild in 72(17.64%) patients, moderate 96(23.52%), severe 98(24.01%) and critical in 89(21.81%), while no change was seen in 53(12.99%) cases (Table 1).

The asymptomatic patients had a median age of 30 years (IQR: 21-55), mild had median age 51 years (IQR: 21-67 years), moderate had median age 50 years (IQR: 17-70 years), severe had median age 51 years (IQR: 28-70 years), and critical had median age 64 years (IQR: 40-84 years) (Table 2).

Azithromycin was the most used drug 304(74.50%), while antiviral Remdesivir was given to 279(68.38%) patients and hydrocortisone to 143(35.04%). Plasma treatment was given to 55(13.48) patients and mechanical ventilation to 87(21.32). Severity

Table-1: Characteristic of patients at admission.

Characteristic	Normal	Abnormal	Total	*p*-value
Total n(%)	161(39.46)	247(60.53)	408	
Gender n (%)				
Male	36(22.36)	179(72.46)	215(52.69)	0.001
Female	125(77.63)	68(27.53)	193(47.30)	0.001
Age Median (IQR)	50(21-76)	64(40-84)	55(18-84)	0.001**
18-29	13(8.07)	27(10.93)	40(9.80)	0.009
30-39	35(21.73)	23(9.31)	58(14.21)	0.007
40-49	27(16.77)	45(18.21)	72(17.64)	0.001**
>50	86(53.41)	152(61.53)	238(58.33)	0.001**
Excluded Comorbidities n (%)	16(1.98)	77(9.56)	93(11.55)	0.512
Diabetes	3(18.75)	16(20.77)	19(20.43)	0.013
Hypertension	2(12.5)	07(9.09	9(9.67)	0.010
Chronic liver diseases	0(0.00)	11(14.28)	11(11.82)	0.033
Ischaemic Heart Diseases	2(12.5)	09(11.68)	11(11.82)	0.001**
Asthma	4(25)	10(12.98	14(15.05)	0.001**
Hepatitis C	1(6.25)	0(0.00)	1(1.07)	0.73
Chronic Pulmonary Obstructive Diseases	4(24)	24(31.16)	28(30.10)	0.001*
Symptoms n (%)				
Asymptomatic	29(18.01)	17(6.88)	46(11.27)	0.003
Fever	26(16.14)	54(21.86)	80(19.60)	0.001**
Cough	38(23.60)	54(21.86)	92(22.54)	0.007
Fatigue	24(14.90)	36(14.57)	60(14.70)	0.001**
Breathing Difficulties	24(14.90)	54(21.86)	78(19.11)	0.001**
Loss of smell and test	20(12.42)	32(12.95)	52(12.74)	0.001
Chest Radiography n (%)				
No Change	44(27.32)	09(3.64)	53(12.99)	0.18
Mild	34(21.11)	38(15.38)	72(17.64)	0.007
Moderate	49(30.43)	47(19.02)	96(23.52)	0.001**
Severe	12(7.45)	86(34.81)	98(24.01)	0.001**
Critical	22(13.66)	67(27.12)	89(21.81)	0.001**
Total Diagnostics Tests				
TLC Median(IQR) Normal:4.0-11.0/10^3/mm^3	7.35(6.4-8.3)	9.98(3.09-16.88)	9.98(3.09-16.88)	0.001**
Lymphocytes Median (IQR) Normal:20-40%	22.5(18-27)	27(5.0-49)	27(5.0-49)	0.372
Platelets Median (IQR) Normal:150-400$10^6$/mm^3	277(165-390)	290(88-492)	290(88-492)	0.475
ALT Median (IQR) Normal:0-43 u/l	18.5(14-23)	30(21-39)	26.5(14-39)	0.001**
AST Median (IQR) Normal:0-40 u/l	19.5(18-21)	27.5(19-36)	27(18-36)	0.001**
ALP Median (IQR) Normal:0-147 iu/l	101(98-113)	146(125-167)	132.5(98-167)	0.001**
Albumin Median (IQR) Normal:34-54 g/l	34.5(41-52)	43.5(31-56)	43.5(31-56)	0.001**
Total Bilirubin Median (IQR) Normal:0.2-1.0 mg/dl	0.67(0.61-0.74)	0.89(0.81-0.97)	0.79(0.61-0.97)	0.001**
GGT Median (IQR) Normal:0-30 IU/L	21(16-26)	23.5(21-26)	21(16-26)	0.001**
Creatinine Median (IQR) Normal:0.60-1.20 mg/dl	0.90(0.87-0.94)	0.93(0.89-0.97)	0.92(0.87-0.97)	0.001**
Urea Median (IQR) Normal:0-50 mg/dl	24(21-27)	47.5(38-57)	39(21-57)	0.001**
CRP Median (IQR) Normal: 0-10/mg/l	10(2.0-18)	29.5(21-38)	20(2.0-38)	0.001**
Ferritin Median (IQR) Normal:M:27-250/F:20-140ng/ml	181(129-233)	272(234-510)	319(129-510)	0.001**
LDH Median (IQR) Normal:14-280 u/l	146(98-194)	351(261-441)	269(98-441)	0.001**
D-Dimer Median (IQR) Normal:100-250 ng/ml	149(90-209)	587(231-944)	517(90-944)	0.001**
CPK Median (IQR) Normal:M:39-308/F:26-192 u/l	117.5(47-188)	224(98-351)	199(47-351)	0.001**

Total number of patients during Admission and Gender is expressed in percentage.. Excluded Comorbidities, initial symptoms, chest Radiography and total diagnostics test estimation is in number and percentage. while individual diagnostics tests and Age data is in the form of Median (IQR) Inter Quartile Range.

TLC= Total Leukocyte Count.ALT= Alanine Aminotransferase.AST= Aspartate Aminotransferase. ALP=Alkaline Phosphatase.GGT= Gamma Glutamyl Transferase.CRP=C-Reactive Protien.LDH=Lactate Dehyderogenase.CPK=Creatine Phosphokinase.T.Bili= Total Bilirubin. The **p- value < 0.001.

Table-2: Diagnostics test results based on diseases severity.

Characteristic	Asymptomatic	Mild	Moderate	Severe	Critical	Total	*p*-value
Severity n(%)	15(3.43)	73(17.89)	124(30.39)	77(18.87)	119(29.42)	408	
Age year/Median (IQR)	30(21-55)	51(21-67)	50(18-70)	51(28-70)	64(40-84)	21(18-84)	
TLC Median(IQR) Normal:4.0-11.0/10^6/mm^3	8.8(7.2-9.2)	8.2(3.70-18.90)	9.0(1.20-87.87)	10.90(1.14-55.60)	14.5(1.26-43.00)	22.07(1.14-43)	0.001
Lymphocytes Median (IQR) Normal:20-40%	23.47(12-30)	39.5(6.0-45)	18(4.0-34)	12(2.0-33.1)	17.5(4-33)	6.0(2.0-33)	0.009
Platelets Median (IQR) Normal:150-400 10^6/mm^3	220(173-450)	182(79-446)	210(86-470)	143(50-360)	245(65-530)	168(50-530)	0.239
ALT Median (IQR) Normal:0-43 u/l	22(12-33)	27(20-40)	42(40-52)	44(21-56)	51(23-72)	44(12-72)	0.001**
AST Median (IQR) Normal:0-40 u/l	23(16-27)	26(17-38)	27(18-35)	43(16-56)	40(36-65)	33(16-65)	0.001**
ALP Median (IQR) Normal:0-147 iu/l	79(56-103)	96(59-134)	138(81-196)	182(103-262)	420(177-664)	155(56-664)	0.001**
Albumin Median (IQR) Normal:34-54 g/l	37(35-44)	34(34-42)	37(30-39)	29(26-33)	27(22-30)	33(22-42)	0.001**
T. Bili Median (IQR) Normal: 0.2-1.0 mg/dl	0.67(0.59-0.72)	0.69(0.62-0.72)	0.83(0.79-0.87)	0.91(0.77-1.09)	1.06(0.91-1.55)	0.81(0.59-1.55)	0.046
GGT Median (IQR) Normal:0-30 IU/L	17.5(12-22)	24(17-28)	28(23-36)	28(23-36)	42(25-55)	24(12-55)	0.001**
Creatinine Median (IQR) Normal:0.60-1.20 mg/dl	0.69(0.68-0.71)	0.79(0.66-0.92)	0.92(0.74-1.11)	1.09(0.92-1.27)	1.24(0.99-1.49)	0.74(0.68-1.49)	0.013
Urea Median (IQR) Normal:0-50 mg/dl	21(16-25)	25(22-39)	31(29-48)	46(31-66)	52(29-79)	42(16-79)	0.001**
CRP Median (IQR) Normal: 0-10/mg/l	12.0(2.0-21)	6.0(0.12-41)	11.25(0.02-547)	23.0(51-1007)	67.0(7.0-1600)	251(2.0-1600)	0.001**
Ferritin Median (IQR) Normal: M:27-250/F:20-140ng/ml	129(123-1420)	200(12-640)	333(17-2417)	435(22-2539)	532(210-3709)	425(17-3709)	0.001**
LDH Median (IQR) Normal:14-280 u/l	127(49-214)	200(22-519)	325(54-1956)	510(127-1037)	470(76-2200)	170(22-2200)	0.001**
D-Dimer Median (IQR) Normal:100-250 ng/ml	118(48-193)	179(22-1100)	272(28-826)	325(120-2000)	1050(159-2231)	176(22-2231)	0.001**
CPK Median (IQR) Normal: M:39-308/F:26-192 u/l	115(31-199)	237(66-408)	422(55-789)	603(198-1009)	743(209-1278)	204(31-1278)	0.001**

During hospitalization individual diagnostics tests data is in the form of Median (IQR) Inter Quartile Range; TLC= Total Leukocyte Count.ALT= Alanine Aminotransferase.AST= Aspartate Aminotransferase. ALP=Alkaline Phosphatase.GGT= Gamma Glutamyl Transferase.CRP=C-Reactive Protien.LDH=Lactate Dehyderogenase.CPK=Creatine Phosphokinase.T.Bili= Total Bilirubin. the ***p*-value < 0.001

level was significantly associated with liver and renal function parameters ($p<0.05$). The use of drug induced various laboratory parameters depending on antibiotics, anti-virals, immune suppressants and anti-coagulants ($p<0.001$)[13] except in some antibiotics, such as moxifloxacin ($p=0.31$), levofloxacin ($p=0.12$) and cefixime ($p=0.22$). Overall, 47(11.51%) patients died (Table 3).

In addition to respiratory failure, 39(70.90%) of the deceased had other complications, including liver failure 1(1.81%), septic shock 26(47.27%), heart failure 13(23.63%), renal failure 2(3.63%) and disseminated intravascular coagulation 12(21.81%) Mortality was the outcome at various stages of the disease progression (Figure 2).

LYM morphology was scored according to the devised scoring system (Table 4).

Discussion

Most deaths in the current study were related to those in the critical phase of the COVID-19 infection. The risk factors identified by the study are similar to those reported earlier.[14-18]

Since COVID-19 has no effective cure, delaying the progression of the disease is essential for survival. Various blood parameters, such as CRP, LDH, ferritin, CPK and D-Dimer, but mostly low LYM with reactive and apoptotic morphology, were identified as parameters marking disease progression. Patients who died tended to have very low levels of LYM count <8% with an apoptotic and reactive morphology, and were 3-times higher risk of death compared to the rest. This corresponds with previous reports.[18,19]

Table-3: Patient outcomes their association with diseases severity.

Characteristic	Asymptomatic	Mild	Moderate	Severe	Critical	Total	*p*-value
Severity n (%)	15(3.43)	73(17.89)	124(30.39)	77(18.87)	119(29.42)	408	
Demographic Characteristic							
Smoking history	3(20)	23(31.50)	25(20.16)	12(15.58)	09(7.96)	72(17.64)	0.472
Respiratory rate >20%	3(20)	13(17.80)	31(25.0)	30(38.9)	79(66.38)	156(38.23)	0.001**
Pulse rate >100%	2(13.3)	11(15.06)	27(21.77)	26(33.76)	68(57.14)	134(32.84)	0.001**
Systolic Blood pressure >140 %	3(20)	18(24.65)	33(26.61)	27(35.06)	66(55.46)	147(36.02)	0.001**
Diastolic Blood pressure >90 %	2(13.3)	17(23.28)	32(25.80)	27(35.06)	49(41.17)	127(31.12)	0.211
Body temperature 1QR 37ºc	36.5(36.0-36.3)	36.8(36.2-36.9)	37.5(37.2-38.1)	37.9(36.9-39.1)	38.5(37.5-40.6)	37.5(36.0-40.6)	0.024
Treatment, use n (%)							
Antibiotics							
Ceftriaxone	5(33.33)	18(24.65)	83(66.93)	26(33.76)	41(34.45)	173(42.41)	0.001**
Moxifloxacin	0(0.0)	0(0.0)	5(4.03)	8(10.38)	5(4.20)	18(4.41)	0.31
Cefixime	0(0.0)	0(0.0)	7(5.64)	2(2.59)	0(0.0)	9(2.20)	0.22
Azithromycin	8(53.33)	62(84.93)	99(79.83)	60(77.92)	73(61.34)	304(74.50)	0.001**
Meropenem	0(0.0)	0(0.0)	0(0.0)	26(6.41)	23(19.32)	49(12.06)	0.001**
Levofloxacin	0(0.0)	0(0.0)	2(1.61)	0(0.0)	5(4.20)	7(1.71)	0.120
Piperacillin And Tazobactam	0(0.0)	0(0.0)	2(1.61)	5(33.76)	39(32.77)	46(11.27)	0.001**
Steroids							
Hydrocortisone	0(0.0)	7(9.58)	49(39.51)	33(42.85)	54(13.46)	143(35.04)	0.001**
Dexamethasone	0(0.0)	10(13.69)	39(31.45)	28(36.36)	60(45.37)	137(33.57)	0.001**
Immunosuppressive							
Tocilizumab	0(0.0)	0(0.0)	0(0.0)	2(2.59)	26(21.84)	28(6.86)	0.001**
Antivirals							
Remdesivir	0(0.0)	47(64.38)	79(63.70)	53(68.83)	91(76.47)	279(68.38)	0.001**
Anti coagulant							
Enoxaparin sodium	0(0.0)	15(20.54)	47(37.90)	65(84.41)	104(87.39)	231(56.61)	0.001**
Plasma therapy	0(0.0)	0(0.0)	29(23.38)	15(19.48)	11(9.24)	55(13.48)	0.003
Mechanical ventilation	0(0.0)	0(0.0)	29(23.38)	15(19.48)	43(36.13)	87(21.32)	0.003
Intravenous immunoglobulin	0(0.0)	0(0.0)	13(10.48)	19(24.67)	21(17.64)	53(12.99)	0.001**
Oxygenation	0(0.0)	21(28.76)	29(23.38)	44(57.14)	11(9.24)	105(25.73)	0.002
Multiple organ failures n(%)	0(0.0)	0(0.00)	11(8.87)	15(19.48)	29(24.36)	55(13.48)	0.001**
Respiratory failure	0(0.0)	1(1.81)	5(9.09)	7(12.72)	26(47.27)	39(70.90)	0.001**
Liver failure	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.81)	1(1.81)	0.031
Septic shock	0(0.0)	2(3.63)	5(9.09)	8(14.54)	11(20)	26(47.27)	0.001**
Heart failure	0(0.0)	0(0.0)	1(1.81)	5(9.09)	7(12.72)	13(23.63)	0.001**
Renal failure	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(3.63)	2(3.63)	0.61
Disseminated intravascular coagulation	0(0.0)	0(0.0)	0(0.0)	3(5.45)	9(16.36)	12(21.81)	0.001**
Clinical out comes n(%)							
Discharged	15(3.67)	70(17.15)	119(29.16)	62(15.19)	95(23.48)	361(88.48)	0.001**
Deceased	0(0.0)	3(0.73)	5(1.22)	15(3.67)	24(5.88)	47(11.51)	0.001**

Table-4: Lymphocytes morphology based on disease severity.

Characteristic		Asymptomatic	Mild	Moderate	Severe	Critical	Deceased	discharged	*p*-value
Severity n(%)		15(3.43)	73(17.89)	124(30.39)	77(18.87)	119(29.42)	47(11.51)	361(88.48)	
Grades	**Lymphocytes Morphology**								
1	1-25 % Reactive Lymphocytes	15(3.43)	22(30.13)	14(11.29)	0(0.00)	0(0.00)	0(0.00)	51(14.12)	0.001**
	1-25 % Apoptotic Lymphocytes	0(0.00)	0(0.00)	9(7.25)	16(20.77)	29(24.36)	0(0.00)	54(14.95)	0.001**
2	25-50% Reactive Lymphocytes	0(0.00)	51(69.86)	87(70.16)	20(25.97)	16(13.44)	0(0.00)	174(48.19)	0.001**
	25-50% Apoptotic Lymphocytes	0(0.00)	0(0.00)	0(0.00)	27(35.06)	34(28.57)	10(21.27)	51(14.12)	0.001**
3	50-75%Reactive Lymphocytes	0(0.00)	0(0.00)	23(18.54)	33(42.85)	78(65.54)	15(31.91)	119(32.96)	0.001**
	50-75% Apoptotic Lymphocytes	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0.003
4	75-100%Reactive Lymphocytes	0(0.00)	0(0.00)	0(0.00)	24(31.16)	25(21.00)	32(68.08)	17(4.70)	0.001
	75-100% Apoptotic Lymphocytes	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	24(51.06)	0(0.00)	0.002

Individual diagnostics test data is collected during hospitalization in the form of a total number and a percentage; In a blood smear, two types of morphological lymphocytes were seen; 1: reactive lymphocytes: Immunoblast-like have big cells with high nuclear-cytoplasmic ratios, compacted chromatin, and profoundly basophilic cytoplasm. Another kind of reactive lymphocyte has less condensed chromatin and copious light blue cytoplasm, giving the appearance of "huggling" neighbouring red blood cells; 2: Apoptotic lymphocytes: Exhibit a number of common morphological characteristics, including cell shrinkage, fragmentation into membrane-bound apoptotic particles, and fast phagocytosis by surrounding cells; p-value < 0.05 was considered statistically significant. the **p-value < 0.001.

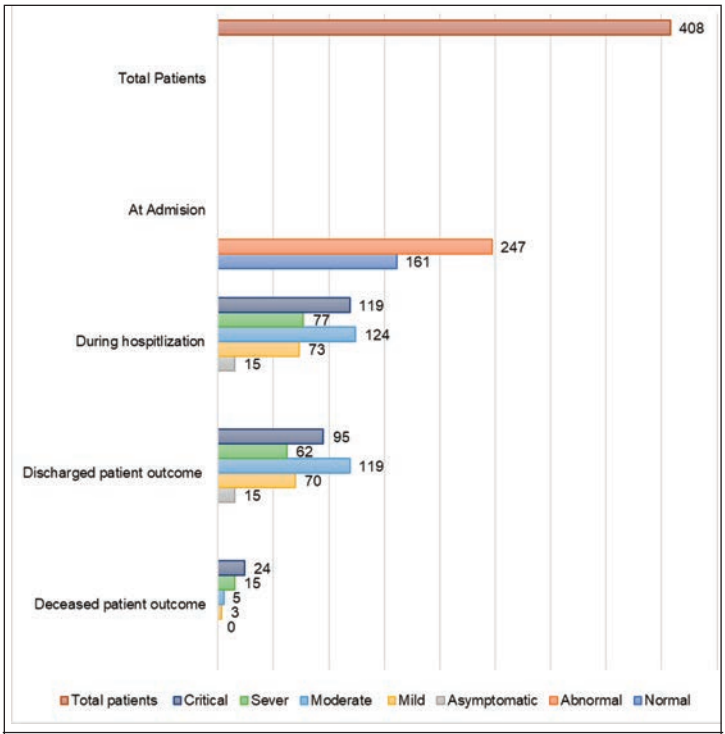

Figure-2: Disease severity and patient outcomes.

Those who died developed multiple organ failure during hospitalisation, and in 85% cases LYM had abnormal morphology. As such, the current data suggests that LYM count with morphology is directly proportional to COVID-19 severity. Elevated blood CRP, LDH, ferritin, CPK, D-Dimer levels also substantially contributed to multiple organ failure in the deceased. The most common was respiratory failure (70.90%), while other systems organs included septic shock (47.27%), heart failure (23.63%), disseminated intravascular coagulation (DIC) (21.81%), liver failure (1.81%) and renal failure (3.63%). Besides, liver damage or dysfunction with lower albumin level and high direct bilirubin have also been reported.[16]

The severity of tissue damage is predicted by LDH. A large amount of it is in circulation when lung tissues are damaged by SARS-COV-2 infection. This often occurs clinically as a severe form of interstitial pneumonia and then develops into acute respiratory distress syndrome (ARDS). Higher LDH levels are also an endothelial predictor, which leads to and is associated with microvascular thrombosis.[20] Therefore, maintaining an effective systemic immune response to multi-organ failure and preventing it is a priority in the treatment of COVID-19 patients.

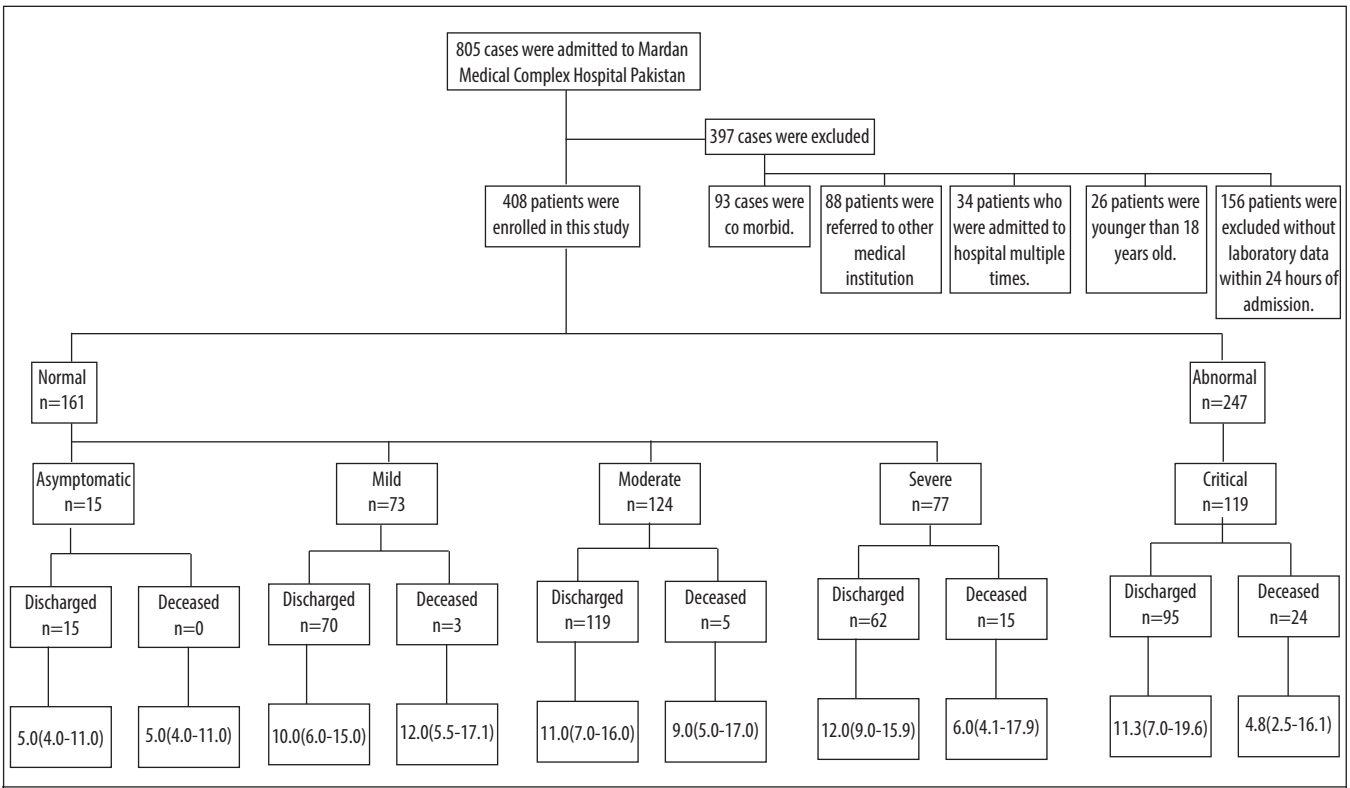

Figure-3: Flow-chart of the study and disease progression.

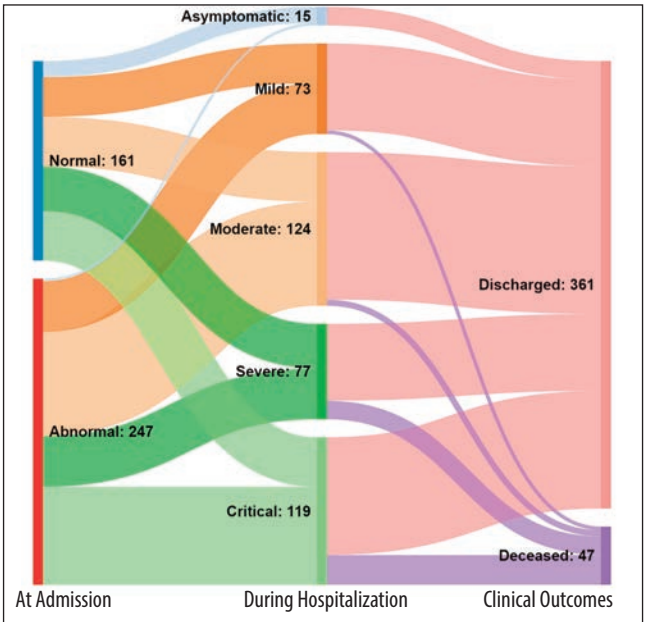

Figure-4: Sankey diagram showing progression in terms of admission, hospitalisation and clinical outcomes.

Based on the current results, CRP, LDH, ferritin, CPK, D-Dimer and morphologically low LYM count are very good predictive biomarkers for accuracy in COVID-19 cases. The liver function and renal function tests did not keep track of disease progression. The change in values related to liver and functions during the hospitalisation were linked to the different medications used.

There are various limitations to the current study. To begin with, it was a retrospective single-centre research and nearly half of the patients were excluded on various grounds that were part of the exclusion criteria. As such, the role of the excluded markers may have been underestimated in the prediction of disease progression and fatality.

Conclusion

At different age and disease severity levels, elevated CRP, LDH, ferritin, CPK, D-Dimer and a decreased number of LYM with reactive and apoptotic morphology were able to track COVID-19 progression. Disease progression and mortality were predicted using a combination of clinical markers linked with systemic responses and multiple-organ failure.

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