

Deciphering the Upshots of Single and Second Dose of ChAdOx1-S Vaccination among Patients Hospitalized with COVID-19: A Multidimensional Approach

Mohd Younus Shah¹, Waseem Feroze Bhat², Harminder Singh³, Gurjinder Singh⁴, Mohd Yunis Saleem Bhat⁵, Kumudini Borole⁶, Tariq PARvaiz Azad⁷

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a dangerous infection throughout the world, despite the availability of affordable and effective management, remaining one of the major causes of death from a single infectious agent worldwide. To mitigate the backlash of the virus on public health the effect of single and second dose of ChAdOx1-S (Verity Pharmaceuticals Inc/Serum Institute of India, in partnership with AstraZeneca Canada Inc) vaccination or covishield has been advocated. It has been elucidated via different biochemical (LFT, KFT, and glucose) hematological (CBC) and antibody analysis (antibodies to SARS-CoV-2 IgG) to gain the outcome of single and second dose. Our study also provides empirical estimates of the effect of vaccination on the different age groups by estimation of values of these tests. The present study shows that simple, inexpensive, highly significant and specific tests should be employed routinely to check the etiology in patients of covid-19. This finding could be helpful in early clinical decision-making for the management of these patients.

Keywords: Antibodies; Biochemical; Coronavirus 2 (SARS-CoV-2); Hematological; Vaccination;

INTRODUCTION

Coronavirus disease (COVID) is an infectious disease caused by Coronaviruses (CoVs). CoVs are a group of RNA viruses that cause illness ranging from the common cold to more severe diseases. In humans most affected organs are the lungs and disease can be mild to life threatening, mild symptoms causing CoVs are hCoV-229E, OC43, NL63, and HKU1, while lethal ones are severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and the novel coronavirus (2019-nCoV) is a new strain that has not been previously identified in humans.¹ World Health Organisation (WHO) named the novel coronavirus "Covid-19". "Co" stands for "corona", "vi" for "virus" and "d" for "disease", while "19" was for the year, as the outbreak was first identified on 31 December 2019. From microscopic point of view CoVs are spherical enveloped by membrane that contains projections of proteins called spike (S) and also in some variants small protrusions of hemagglutinin-esterase (HE) protein is found (figure 01).²⁻³ These protein projections look like sun's corona and virus gets the name coronavirus. Genetic material is mainly RNA inside the envelope. Coronavirus make people sick, usually with a mild to

moderate upper respiratory tract illness, similar to a common cold. Some of the symptoms are: runny nose, sore throat, headache, cough and fever. In some cases, such as pneumonia or bronchitis occurs which needs immediate professional health consultation.⁴ At the beginning such as hand washing, social distancing, and masks were recommended to prevent the spread of Covid-19, although these efforts has resulted scanty success and is based on implementation efforts. However, due to hike in the daily reports of Covid-19 cases worldwide a global urgency for vaccine development has been came into existence. So many countries developed Covid-19 vaccines under different names like, Oxford-AstraZeneca's COVID-19 vaccine (viral vector vaccine) by UK, The Janssen Ad26.COVS2.S COVID-19 vaccine (viral vector vaccine) by USA, The Moderna COVID-19 (mRNA-1273) vaccine by USA, The Pfizer BioNTech (BNT162b2) COVID-19 vaccine (RNA vaccine) by Germany, The Sinovac-CoronaVac COVID-19 vaccine (inactivated vaccine) by China, The Bharat Biotech BBV152 COVAXIN vaccine (whole inactivated virus) by India, The Novavax vaccine, The CanSino Biologics Ad5-nCoV-S COVID-19 vaccine (viral vector vaccine) by China, and many others by different countries.⁵ Here we have seen the effect of single and second dose of ChAdOx1-S (Verity Pharmaceuticals Inc/Serum Institute of India, in partnership with AstraZeneca Canada Inc) vaccination or covishield on the Chenab valley people hospitalized in government medical college (GMC) Doda, India during 2021 and 2022.

¹Professor and HOD, Department of Medicine, GMC, Doda,

²Demonstrator, Department of Biochemistry, GMC, Doda,

³Associate Professor, Department of Medicine, NCMC Panipat,

⁴Asst Professor, Department of Biochemistry, al Falah Medical

university, Haryana, ⁵Professor, Department of Dentistry, GMC,

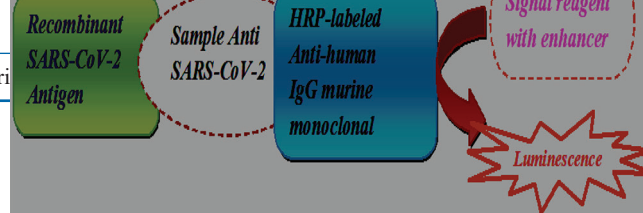
Doda, ⁶Professor, Department of Biochemistry, GMC, Doda,

⁷Professor, Department of Surgery, GMC, Doda

Corresponding author: Dr. Waseem Feroze Bhat, Department of Biochemistry, Government Medical College Doda, Jammu and Kashmir, India- 182202

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MATERIALS AND METHODS

The present study was conducted on Covid-19 patients admitted in medicine ward government medical college (GMC) Doda and the tests were performed in the department of biochemistry GMC Doda during April 2021 and upto April 2022. The various biochemical investigations of these patients were assessed and compared to the normal controls. The antibodies were checked, Liver function tests (LFT), kidney function tests (KFT), Complete blood count (CBC), Rapid Kit and Real-Time Reverse Transcription–Polymerase Chain Reaction (RT-PCR) were assayed in both control and covid-19 patients. The age groups of all the persons from less than 10 years to above 60 years were taken. Both the gender was taken table 01.

649 covid-19 positive symptomatic patients were admitted from April 2021 to April 2022. Patients were categorized into four groups, namely, the single dose, second dose, symptoms presentation, and number of days stayed in hospital.

Methods

Different tests were performed by employing various methods.

Rapid Kit and RT-PCR test

Rapid test for qualitative detection of novel corona virus antigen in human nasopharyngeal swab specimen by using OS KIT (Oscar Medicare Pvt. Ltd.). RT-PCR was done by commercial ready-to-use Meril COVID-19 one step RT-PCR Kit (manufactured by Meril) and RNA extraction was done by GeneMag Viral DNA/RNA Purification Kit (The GeneMag Viral DNA/RNA Purification Kit is highly designed for rapid purification of high quality nucleic acid from virus in samples such as swabs, saliva, blood, bodily fluid, plasma/serum, urine, and viral transport media), as indicated in table 02.

High-resolution computed tomography (HRCT)

HRCT scans were performed at radiology department GMC Doda, using the TOSHIBA SCANNER, MODEL TSX-032A, toshiba medical systems Europe. Desired patients were shifted to the radiology department and their chest was scanned under proper medical care. Prior to shifting in the ward, individuals with symptoms of COVID-19 were undergoing different COVID-19 testing and the individuals with the severity of the disease were shifted to radiology department for HRCT after COVID-19 testing. Below table 02 indicates the total number of individuals and their COVID-19 testing with different methods.

Test for Antibodies

Serum of desired samples were checked for the antibodies to SARS-CoV-2 IgG using quantitative SARS-CoV-2 chemiluminescent microparticle immunoassay (CLIA) on Ortho Clinical Diagnostics VITROS® ECiQ Immunodiagnostic System (Johnson & Johnson) with proprietary enhanced chemiluminescence detection technology provides wide dynamic ranges and exceptional precision and sensitivity.

Test type and conditions for antibodies

Test type:	Immunometric
System:	Chemiluminescence detection technology (ECiQ)
Incubation Time:	37 Minutes
Time of result:	48 Minutes
Blood collection vacutainer:	Red Top
Temperature:	37°C
Reaction sample volume:	20µL (Serum)
VITROS Anti-SARS-CoV-2 IgG	Specimen is non-reactive (negative) for anti-SARS-CoV-2 IgG
test result (<1.00):	
VITROS Anti-SARS-CoV-2 IgG	Specimen is reactive (positive) for anti-SARSCoV-2 IgG
test result (≥1.00):	

Test for CBC

A complete blood count (CBC) is a common laboratory investigation that is used in a wide range of conditions. Blood of desired covid-19 patients were drawn from the cubital vein into appropriate blood collection tubes using vacuum tube needles. K2EDTA tubes were used for CBC analyses. BC-5130 Auto Hematology analyzer A “CUTE” 5-part Shenzhen Mindray Bio-Medical Electronics was used.

Test type and conditions

Test type:	Impedance method for RBC and PLT counting. Cyanide free reagent for hemoglobin test. Flow Cytometry +Tri-angle laser scatter + Chemical dye method for WBC counting.
System:	BC-5130 Auto hematology analyzer
Blood collection vacutainer:	Lavender top
Approximate rotation time:	10 Minutes
Temperature:	37°C
Reaction sample volume:	2 ml (Whole blood)

Test for KFT

KFT parameters like Creatinine and Urea of covid-19 patients were measured quantitatively by using world class analyzer

equipped with double beam photometer, fully automatic biochemistry analyzer (Metrolab 2300 plus) compatible with analysis method like end-point, fixed time, and kinetic.

Test type and conditions

Test type:	Colorimetric (Jaffe Method, Rate Kinetic)
System:	True double beam optical system (Metrolab 2300 PLUS).
Blood collection vacutainer:	Red top
Temperature:	37°C
Wavelength:	505, nm
Reaction sample volume:	10 µl (Serum)

Test for LFT

LFT parameters like alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), albumin and total protein of covid-19 patients were measured quantitatively by using world class analyzer equipped with double beam photometer, fully automatic biochemistry analyzer (Metrolab 2300 plus) compatible with analysis method like end-point, fixed time, and kinetic.

Test type and conditions

Test type:	Colorimetric (UV-Kinetic method)
System:	True double beam optical system (Metrolab 2300 PLUS).
Blood collection vacutainer:	Red top
Temperature:	37°C
Wavelength:	340,405 and 600 nm
Reaction sample volume:	10 µl (Serum)

Test for glucose

Glucose concentration of covid-19 patients were measured quantitatively by using world class analyzer equipped with double beam photometer, fully automatic biochemistry analyzer (Metrolab 2300 plus) compatible with analysis method like end-point, fixed time, and kinetic.

Test type and conditions for glucose

Test type:	Colorimetric (End point method)
System:	True double beam optical system (Metrolab 2300 PLUS).
Approximate incubation time:	10 minutes
Blood collection vacutainer:	Grey top
Temperature:	37°C
Wavelength:	540 nm
Reaction sample volume:	10 µl (Serum)

STATISTICAL ANALYSIS

The descriptive data were summarized as the mean, median, standard deviation, Std. Error Mean, Mean Difference, T value and P value. Mean levels of different parameters were compared between vaccinated individuals with and without vaccination. The data was obtained and entered in Microsoft Excel Version 13 and was subjected to statistical analysis using IBM SPSS Version 21. These were subjected to intragroup comparison between different age groups and among two age groups below 60 and above 60 years, which was performed using ANOVA. Analysis of Variance was conducted between Groups keeping confidence interval at 95% and ($p < 0.05$) was considered to be statistically significant.



RESULTS

The study involved patients with a confirmed diagnosis of COVID-19 admitted to GMC Doda between April 2021 to April 2022. 649 covid-19 positive symptomatic patients were admitted. Patients were included without vaccinated, single dose and double dose vaccinated. Second and first dose vaccinated patients were not previously infected. The study protocol was approved by the GMC Review Board. Consent was taken from the respective departments for the nature of the study.

The study included 341 females and 308 males. These were further grouped according to age as shown in table 01. Different departments were involved in performing the different tests. The various biochemical investigations of these patients were assessed and compared to the normal controls.

Level of Covid-19 antibodies

We checked for the antibodies to SARS-CoV-2 IgG and compared mean levels of SARS-CoV-2 IgG, in the groups based on COVID-19 natural infection status (without vaccination). The mean levels of SARS-CoV-2 IgG, antibodies were higher in the groups those took second shot of vaccination the level reached (above 20 AU), than those groups who took only first shot of vaccination, but were less (below 14 AU) in those who were infected and did not undergo for vaccination as depicted in figure 02 (a), (b), and (c). In the context of present study, patients who were aged between 20 to 30 years were able to develop the immunity for Covid-19 very fast and produce the antibodies to somehow very high as compared to higher age groups. As depicted in Fig. 02 (a) the value of Anti-SARS-CoV-2 IgG test result reached the maximum for the age group upto 20 years and value reduced for higher age group same trend was seen in Fig. 02 (b). However, the value for Anti-SARS-

CoV-2 IgG test result was very less in Fig. 02 (c) and the trend was same reaching maximum for age group 20 and reduced for higher age group. The mean Anti-SARS-CoV-2 IgG test result for second/single/without dose depicted that there was a decrease in values as the age increased and the difference in mean was statistically significant $p < 0.03/p < 0.00/p < 0.00$. It could be summarized that the antibodies were formed maximum in patients who were vaccinated and were naturally infected. Below 10 years of age all were without dose and Anti-SARS-CoV-2 IgG test was not performed for

them (table 03). Normal individual without vaccination and infection was treated as control (normal) and the level was checked for Anti-SARS-CoV-2 IgG which came zero.

Association of antibody among two age groups and COVID-19 disease severity

It has been observed that above 60 years of age patients have been stayed longer days with more severity of disease and took longer time to recover (table 03). The production of vaccine-induced antibodies was significantly higher ($p < 0.000$) among the patients aged below 60 years (table 04) with more profound effect in second dose individuals aged below 40 years. However, second dose individuals aged above 60 years develop antibodies more as compared to single dose.

It could be concluded that individuals those got two doses of the ChAdOx1-S vaccine and were infected with SARS-CoV-2 had a significantly higher levels of antibodies compared to individuals who were infected with SARS-CoV-2, but were not vaccinated. An interesting trend has been noticed that natural infection produced antibodies which were declined very slowly and lasting for more time. But individuals with vaccination and were not undergo natural infection produced

Gender

Males	308
Females	341
Age (Years)	
Less than 10	15
11 to 20	23
21 to 30	66
31 to 40	76
41 to 60	200
Above 60	269

Table-1: Details of the covid-19 positive symptomatic patients

Age (Years)	Total Individuals	Rapid Kit	RTPCR	HRTC
≤10	15	10	05	0
11 to 20	23	17	06	02
21 to 30	66	48	18	05
31 to 40	76	46	30	09
40 to 60	200	117	83	17
≥ 60	269	155	114	37

Table-2: Details of the covid-19 testing by employing RTPCR and Rapid Kit method.

Age (Years)	N	Anti-SARS-CoV-2 IgG test result (Average)	No. of days stayed in hospital (Average)	Severity of disease
(a) Without dose				
≤10	All	Not performed	03-05 (Days)	Mild, Moderate
11 to 20	17	Reactive (positive)	03-05 (Days)	Moderate, Unstable
21 to 30	50	Reactive (positive)	03-08 (Days)	Moderate, Unstable
31 to 40	60	Reactive (positive)	05-10 (Days)	Moderate, Unstable, Severe
40 to 60	130	Reactive (positive)	05-12 (Days)	Unstable, Severe
≥ 60	129	Reactive (positive)	05-14 (Days)	Unstable, Severe
(b) Single dose				
≤10	Nil	Not performed	Nil	Nil
11 to 20	3	Reactive (positive)	02-03 (Days)	Moderate
21 to 30	5	Reactive (positive)	02-03 (Days)	Moderate, Unstable
31 to 40	8	Reactive (positive)	02-05 (Days)	Unstable
40 to 60	45	Reactive (positive)	02-07 (Days)	Unstable, Severe
≥ 60	75	Reactive (positive)	05-08 (Days)	Unstable, Severe
(c) Second dose				
≤10	Nil	Not performed	Nil	Nil
11 to 20	3	Reactive (positive)	02-03 (Days)	Moderate, Unstable
21 to 30	11	Reactive (positive)	02-03 (Days)	Moderate, Unstable
31 to 40	8	Reactive (positive)	02-03 (Days)	Moderate, Unstable
40 to 60	25	Reactive (positive)	02-05 (Days)	Moderate, Severe
≥ 60	65	Reactive (positive)	03-07 (Days)	Moderate, Severe

Table-3: Shows the severity of disease, No. of days stayed in hospital and the Anti-SARS-CoV-2 IgG test result among the different aged groups patients having double (c), single (b) and without vaccination (a).

antibodies which slumped very fast and endured for less time.

CBC analysis

Complete blood count (CBC) reference is an important for

the diagnosis of different diseases, screen blood donors, and assess overall health. CBC analyses have been done almost moderate to severe symptomatic patients. However, current study established and monitored the White blood

Age Group	N	Mean	Std. Deviation	T Value	P Value
(a) Without dose					
Below 60 Years	257	6.9105	1.89463	11.635	0.000
Above 60 Years	129	4.8295	1.03172		
(b) Single dose					
Below 60 Years	61	9.2623	2.89771	6.892	0.000
Above 60 Years	75	6.5067	1.71143		
(c) Second dose					
Below 60 Years	47	12.6809	3.57592	9.698	0.000
Above 60 Years	65	7.9692	1.36896		

Table-4: Shows the Anti-SARS-CoV-2 IgG test result among the two groups below 60 years and above 60 years showing p and t value, (a) Without vaccination, (b) Single dose, (c) Double dose

Age (Years)	N	WBC (3.50-9.50 x10 ³ /μL) (Mean±SD)	Thrombocytes (125-350 x10 ³ /μL) (Mean±SD)	HB (11.5-17.5 g/dl) (Mean±SD)
(a) Without dose				
≤10	15	8.05±S1.15	226.33±42.15	12.96±1.15
11 to 20	17	8.81±1.12	230.88±43.56	13.17±1.14
21 to 30	50	9.41±1.08	222.90±39.05	13.62±1.55
31 to 40	60	9.32±0.83	210.33±36.69	12.70±1.30
40 to 60	130	8.31±1.23	183.11±39.83	11.90±1.27
≥ 60	129	4.16±0.95	150.42±35.34	11.00±1.33
(b) Single dose				
≤10	Nil	-	-	-
11 to 20	3	8.18±1.64	230.00±65.57	14.00±1.00
21 to 30	5	8.80±1.35	243.00±53.57	14.40±1.08
31 to 40	8	7.59±1.26	208.12±44.07	13.50±1.00
40 to 60	45	7.31±0.81	185.77±33.35	12.18±1.24
≥ 60	75	4.40±0.97	157.41±34.57	11.76±1.20
(c) Second dose				
≤10	Nil	-	-	-
11 to 20	3	7.92±1.11	246.67±50.33	14.00±1.00
21 to 30	11	8.05±0.91	240.45±33.04	14.13±1.02
31 to 40	8	7.33±1.20	214.37±32.45	13.75±0.92
40 to 60	25	6.80±0.91	199.00±29.65	12.08±1.24
≥ 60	65	4.73±0.96	167.71±28.60	11.83±1.17

Table-5: WBC, Platelet count and, HB changes among the covid-19 positive patients (a) without dose, (b) single dose, (c) second dose of hospitalized COVID-19 patients

Age Group	N	Mean	Std. Deviation	T Value	P Value
(a) Without dose					
Below 60 Years	272	8.7638	1.24937	36.928	0.000
Above 60 Years	129	4.1690	0.95817		
(b) Single dose					
Below 60 Years	61	7.5184	1.03795	18.014	0.000
Above 60 Years	75	4.4040	0.97321		
(c) Second dose					
Below 60 Years	47	7.2579	1.09016	12.924	0.000
Above 60 Years	65	4.7385	0.96302		

Table-6: Shows the WBC count test result among the two groups below 60 years and above 60 years showing p and t value, (a) Without vaccination, (b) Single dose, (c) Double dose.

Age Group	N	Mean	Std. Deviation	T Value	P Value
(a) Without dose					
Below 60 Years	272	201.8015	43.41371	11.721	0.000
Above 60 Years	129	150.4264	35.34999		
(b) Single dose					
Below 60 Years	61	195.8197	41.37411	5.897	0.000
Above 60 Years	75	157.4133	34.57731		
(c) Second dose					
Below 60 Years	47	214.3617	36.40976	7.590	0.000
Above 60 Years	65	167.7154	28.60329		

Table-7: Shows the platelet count test result among the two groups below 60 years and above 60 years showing p and t value, (a) Without vaccination, (b) Single dose, (c) Double dose.

Age Group	N	Mean	Std. Deviation	T Value	P Value
(a) Without dose					
Below 60 Years	272	12.5368	1.47567	10.018	0.000
Above 60 Years	129	11.0039	1.33243		
(b) Single dose					
Below 60 Years	61	12.6311	1.40506	3.864	0.000
Above 60 Years	75	11.7667	1.20341		
(c) Second dose					
Below 60 Years	47	12.9681	1.46482	4.522	0.000
Above 60 Years	65	11.8385	1.17629		

Table-8: Shows the HB concentration test result among the two groups below 60 years and above 60 years showing p and t value, (a) Without vaccination, (b) Single dose, (c) Double dose.

Age (Years)	N	AST (05-40U/L) (Mean±SD)	ALT (07-50U/L) (Mean±SD)	ALP (60-298U/L) (Mean±SD)	TBIL (0.1 to 1.2 mg/dl) (Mean±SD)
Without dose					
≤10	15	19.40±6.64	20.26±6.30	131.33±26.14	0.48±0.18
11 to 20	17	20.29±9.35	22.29±8.46	135.29±27.86	0.54±0.20
21 to 30	50	26.14±9.26	27.56±7.67	144.60±31.44	0.69±0.25
31 to 40	60	32.05±8.55	31.08±9.06	161.66±40.42	0.76±0.23
40 to 60	130	39.87±12.42	39.04±11.73	178.95±49.46	0.78±0.23
≥ 60	129	44.45±17.51	44.40±17.33	210.15±75.08	0.84±0.23

Table-9: Liver function test shows the different types of parameters in without dose patients like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TBIL) of different age group individuals admitted in the hospital.

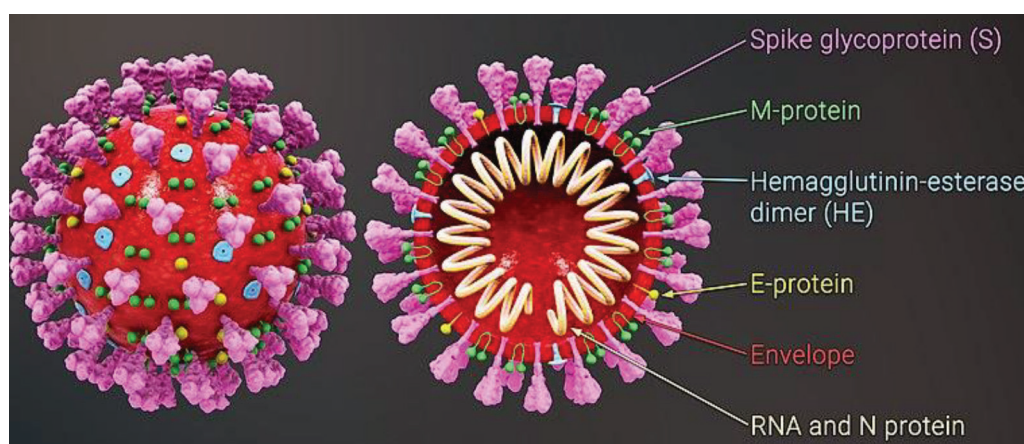


Figure-1: Microscopic image of CoV shows projections of protein (spike) and also in some variants small protrusions of hemagglutinin-esterase dimer.²⁻³

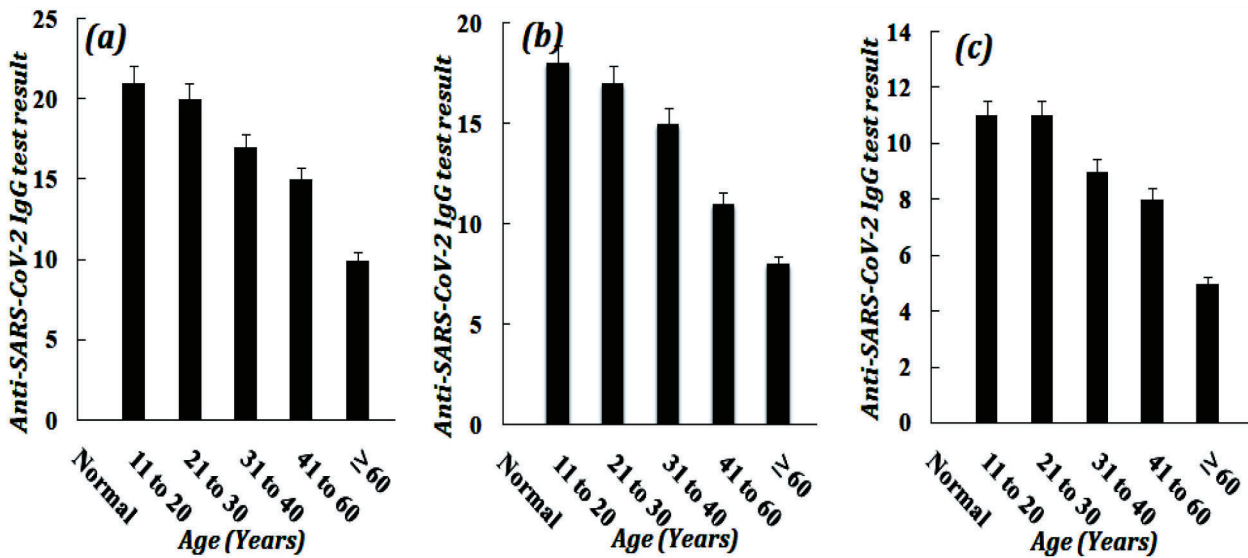


Figure-2: (a) Antibody responses among the different age groups following the second dose of ChAdOx1-S vaccine in uninfected individuals (b) Individuals with single dose and natural infection (c) SARS-COV-2 IgG among the individuals with natural infection and devoid of vaccination among different age groups, although not sharp increment but were long lasting. Error bars represent the percentage error of the test.

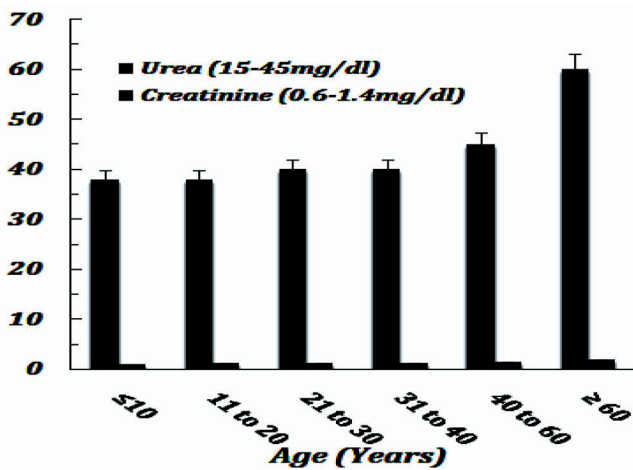


Figure-3: Kidney function analysis shows the Urea and Creatinine values of the individuals with different age groups. Error bars represent the percentage error of the test

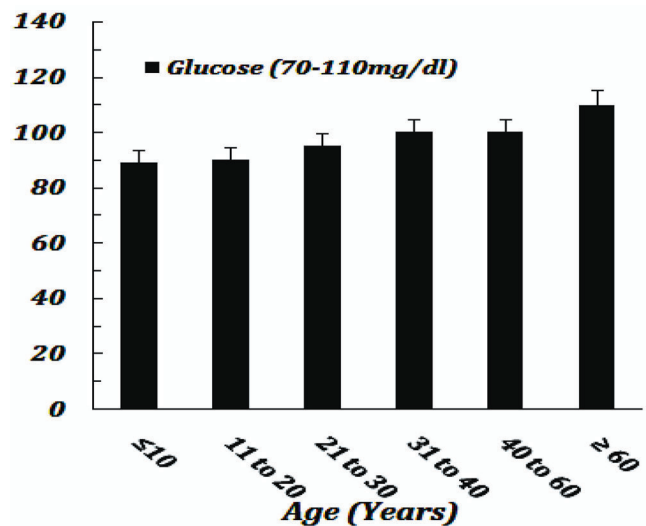


Figure-4: Glucose level changes of different age groups admitted in the hospital with Covid-19 positive infection included vaccinated and non vaccinated patients. Error bars represent the percentage error of the test.

cell (WBC), Hemoglobin (HB) and Platelets (PLT) count for severe and unstable COVID-19 patients as the major changes have been discerned only in the above three parameters. The aim of this analysis was also to distinguish the changes in comorbid patients and their above CBC changes during infection. In general it has noticed that WBC count has been increased during the initial phase of infection which came to normal with the time as patients recovered from the disease. However, platelet count did not decreased and HB reduced in some female patients. Table 05 showed that without dose, patients' up to 40 years of age showed very high increase in WBC count (above than normal) on the initial admitted days, thrombocyte count and HB was very little affected. However; above 60 years of age patients showed abnormal (lower than normal) WBC and platelet count, HB was also decreased in the same aged group patients. Single dose patients showed

variation in the above three parameters, again WBC and thrombocyte count was low among the patients aged above 60 years. However; very less variation in WBC and thrombocyte count was detected in second dose patients also having normal HB. The WBC, Thrombocytes and HB levels for second/single/without dose ($p < 0.03$ / $p < 0.05$ / $p < 0.00$) depicted that as the increased the mean values of these parameters decreased which showed a statistical significance ($p < 0.05$).

Furthermore it has been ascertained that above 60 years of age with second dose showed increase in mean of WBC, thrombocytes count and HB concentration having low T value and $p < 0.05$ as compared to single dose and without dose showed a statistical significance (table 06, 07, 08).

However there was less effect on the age group below 60 years, this indicates that, patients with above 60 years without dose and less probable of single dose are likely to embrace different causes like endothelial damage which can activate platelets, aggregation and thrombosis in the lung, causing vast platelet consumption or may directly infect bone marrow, or trigger an auto-immune response against blood cells.

KFT analysis

Kidney function test was used to determine the urea and creatinine values among the COVID-19 patients. It has been done to observe the effect of covid-19 on the renal function of positive patients. It has been seen that comorbid patient without dose and aged above 60 years of age showed abnormal (higher than normal) urea and creatinine values as shown in figure 03. The mean Urea and Creatinine levels increased as the age increased and this mean urea and creatinine levels increased significantly ($p < 0.05$). The patients less than 60 years of age and devoid of dose showed very small variation in urea however, that has been come to normal with the time as patients recovered from the disease. Patients with single and second dose patients showed normal urea and creatinine values very few exceptional cases of comorbid patients with second dose showed paltry variations in urea and creatinine values.

Liver parameters

The present investigation observed the changes in liver functioning of positive patients. The study emphasis on the data of non-vaccinated, single dose and second dose individuals included males, females and controls. The present study has emulated the investigation that were rational with other results. In general procedure, different parameters of LFT were taken into consideration like total bilirubin (TBIL), alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) of covid-19 patients. TBIL, ALT, AST and ALP with $p < 0.003$, $p < 0.021$, $p < 0.05$, $p < 0.03$ depicted that without dose the mean values increased which portrayed a statistical significance ($p < 0.05$). It has been observed that patients aged group above 60 years with devoid of any dose AST followed by ALT was most deranged while ALP was not much elevated and total bilirubin was least deranged (Table 09). In patients with single dose vaccination liver enzymes were less deranged than without dose patients. Moreover, it has been seen that patients with second dose of vaccination and without comorbidities showed very least liver enzyme abnormalities. However, in comorbid patients deranged liver enzymes have been observed irrespective of any age group.

Blood sugar analysis

Blood sugar is the key component which can be deranged either with pancreatic malfunction or in obesity, although so many metabolic changes with different health conditions like pregnancy or in hyperglycemia glucose level elevated, but with reference to present context glucose estimation has been considered of patients with Covid-19 infection. It was ascertained that no change of blood glucose was occurred in

the all three groups (without dose, single dose and second dose) patients irrespective of age groups. However, already diabetic patients with Covid-19 infection or vaccinated in the comorbid category deranged effect was not found in their glucose level, figure 04 shows that scanty changes in glucose level have been noticed in the elder patients and it was observed very significant in the comorbid patients.

3.6. Follow-up Investigations

After discharged from the hospital recovered patients were advised for follow up checkup and it has been ascertained that accretion of antibodies were found more in natural infection than vaccinated individuals. It is worth to mention here that during follow up checkup slight increase of fasting glucose has been noticed, this has been seen in both without dose and vaccinated individuals, this demonstrated that diabetes may be the concern in very long run for these patients, rest observations were normal. However, slight memory problems like remembering of things have been observed in covid -19 positive patients.

The findings of the study showed a decrease in antibodies after vaccination and infection; thus we need amendments of Covid-19 vaccination to boost a single vaccination strategy so that lifelong or maximum time immunity could be developed.

DISCUSSION

Expedition spread of coronavirus disease (Covid-19), has affected millions of lives since its emergence in December 2019 in Wuhan city China.⁶ It has affected more than 150 countries around the world. Coronavirus causes severe respiratory symptoms, also lead to multiple organ failure (MOF) and even death in severe or critical cases.⁷⁻⁸ Studies have shown that patients infected with SARS-CoV-2 may develop antibodies. However, severe cases with comorbidities were more likely develop renal problems, lymphocytopenia, thrombocytopenia, and liver injury compared to mild cases.⁸⁻⁹

SARS-COVID-19 pathogenesis utilizes the angiotensin converting enzyme-2 (ACE-2) receptor for entering in to the cell. This receptor plays a vital role in the propagation of virus as it is scattered on endothelial cells. Post-mortem outcomes encountered within deceased patients revealed lung injury as depicted in HRCT denoting the less oxygen saturation, moderate microvascular steatosis, mild lobular and portal activity.⁹⁻¹⁰ It is currently uncertain whether the COVID-19-related abnormalities are due mainly to the viral infection or other coexisting conditions, such as the use of potentially hepatotoxic drugs, systemic inflammatory response, angiotensin-converting enzyme (ACE) 2-mediated liver dysfunction, respiratory distress syndrome-induced hypoxia, multiple organ dysfunction, lung problems and other biochemical abnormalities.¹¹

The present study used different approaches to find correlation between different biochemical mechanisms and their fate of end products/biomolecules during the infection of covid-19. Scientifically speaking from immune mechanism to blood dimensions (WBC, AST, ALT, HB etc.) analysis has been

done for the members of admitted patients with covid-19 infection. 70 patients were died having multiple ailments along with Covid-19 may be due to thrombocytopenia, Lymphopenia, low saturation, renal failure or with anemia. Comorbid patients have shown decreasing trend (below normal) in WBC count with reduced HB and usually less than $100 \times 10^3/\mu L$ platelets, these patients were generally aged above 60 years and some were thrombocytopenia and some were anemic. Among these comorbid patients' majority died in the hospital with less PO_2 levels and very high HRCT score. Thrombocytopenia in these patients is likely due to various causes, viral infection and ventilation could trigger endothelial damage which can activate platelets, aggregation and thrombosis in the lung, causing vast platelet consumption¹², CoV may also directly infect bone marrow, or trigger an auto-immune response against blood cells.^{12,13} Similar results were discussed among the Covid-19 patients by Lippi et.al in 2020.¹⁴ Close monitoring of different changes in these indices has helped in understanding the changes in the patient's condition. Study has shown that WBCs are the main target cells of viral infections.¹⁵ Viral infections cause damage to the immune system, which in-turn decrease in the number of lymphocytes.¹⁶ It has been shown that below 60 years and without any other ailments patients were observed with higher number of lymphocytes which is the significant mark of any viral or bacterial infection. However, absolute decrease in the number of lymphocytes has been detected in comorbid and above 60 years patients. There may be multifactor causes those could trigger the reduction of lymphocytes. However, it has been demonstrated that depletion of lymphocytes may favor viral persistency by abrogating T-dependent immune mechanisms involved in the viral elimination process and, thus, lead to the development of a chronic disease and persistency of immunodeficiency¹⁷, and it has been hypothesized that virus could directly infect hematopoietic cells and bone marrow stromal cells via CD13 or CD66a, which induce growth inhibition and apoptosis; and cause an immune damage to the blood system by inducing auto-antibodies and immune complexes.¹⁸ Because of the major adverse effect of such viral infections causes the immunomodulation and comorbid or aged patients undergo sever clinical manifestations which are hardly manageable. A significant decrease of absolute WBCs was also observed in SARS patients, which were also lower than those of the patients infected with human immunodeficiency virus(HIV), Cytomegalovirus(CMV), and Epstein-Barr virus (EBV).¹⁹ It could be stated that manageable treatment for reduction of lymphocytes may be the option to save the life of the covid-19 patient. Although vaccination has significantly managed the loss of lymphocytes and thrombocytes as depicted in the table 05, this signifies the importance of vaccination especially the effect of second dose which in-turn raises the covid antibodies those may play the role in lessening the efficacy of virus and the present study demonstrates that COVID-19 patients should undergo a CBC to monitor the signs of disease progression. The present study further demonstrated the liver enzyme

changes in the same groups and the results demonstrated that patients aged group above 60 years with devoid of any dose AST followed by ALT was most deranged while ALP was not much elevated and total bilirubin was least deranged, these results were rational with other studies.^{20,21} It could not be diagnosed that derangement of liver enzymes have been mainly due to liver injury caused by drug administration during the disease or it could be directly from the virus effect.²² However, comorbid patients were more prone to liver injury and it has been observed during the LFT assay performance. This could be elucidated that during the medicine prescription for the comorbid and old aged patients, it must be synchronized that proper liver enzyme check up to be followed during the course of medicine. In case of comorbid and above 60 years aged individuals to some extent creatinine and urea have been elevated and it could trigger the kidney damage via the uremia. The findings supported that covid-19 also causes severe multi-organ dysfunction in humans.^{23,24} and our results support the same. It has been summarized that being vaccinated and especially second dose vaccination was associated with a lower risk of liver and kidney injury and also less severity of disease has been reported. Thus present study supported continuous efforts to increase vaccine cover in aged and comorbid groups in populations.

As depicted in the figure 02 (a) second dose individuals were having more antibodies than those of single dose, which is also rational with other studies²⁵ as different biochemical and hematological parameters were approachable to normal line especially in penitents with below 60 years. Second dose poses the significant effect in case of their lymphocytopenia, thrombocytopenia or low HB chances to occur if denied to accept the vaccination. Antibodies were more in case of second dose which signifies the fact that persistence of antibodies for longer duration and helped to fight against virus and other pathological abnormalities related with the disease. It is clearly noticed in the tables 03 that No. of days stayed in hospital were less in case of second dose patients and were more for without dose. Severity of disease in case of no dose was very more and most aged patients shown very severe symptoms and majority of deaths have been reported in the same group, as far as second dose patients are concerned severity of disease was less as compared to single and without dose individuals. The study confirms that second dose vaccination results in a stronger antibody response than single dose and without vaccination, our results were rational with other findings.^{25,26} Our data showed antibody rise and thus support in implementing strategies for vaccination policies and different biochemical hematological analysis must be done to curb the future viral or any other pathogenic pandemic (figure 05).

One more biochemical estimation has been done for the admitted patients i.e. glucose estimation and it has been observed that there was no change in fasting glucose level in non diabetic patients. However, during follow up checkup slight increase of fasting glucose has been noticed, this has been seen both in without dose and vaccinated individuals,

but we could not predict in a precise way that it could be the effect of vaccination or natural infection.

CONCLUSION

Treatment of covid-19 positive patient remained a challenge since its outbreak. The present study showed that simple, inexpensive, highly significant and specific tests like CBC, LFT, KFT and antibodies should be employed routinely to diagnose the severity of disease and the effect of vaccination. Vaccination is mainly persuasive and relatively safe and it has prevented the severity of the disease. Beyond this impressive efficacy of COVID-19 vaccines, notably different diagnostic tests must be done to modify the course of management. This finding could be helpful in early clinical decision-making for the management of these patients, as it could lead to a better prognosis and avoidance of potential adverse consequences and alert clinicians to pay attention not only to the symptoms of respiratory dysfunction but also the symptoms of other abnormalities. Such a combined diagnostic technique can ameliorate research efficiency and make the diagnosis easy. The findings of this study suggest that among the general masses, COVID-19 vaccine boosters are important and updated vaccines effective against emerging SARS-CoV-2 variants are needed. Further work is necessary to identify the best (and simplest) combination that will be most useful in clinical practice.

FUTURE OUTCOME

Such a combined experimental approach can improve research efficiency. The outcome of the proposed studies will help design therapeutic strategies to either promote or mimic the present or future medical approach for Anti-SARS-CoV-2 positive patients and to develop vaccine that could protect against different strains (as the virus undergoes rapid mutations) or vaccination programs like smallpox and polio (highly contagious viruses) that were almost eradicated through vaccination.

Declarations

Ethics approval and consent to participate

This study was approved by ethical committee of government medical college Doda (GMCdoda/IEC/2021/25). Informed consent was obtained from all the participants and/or their LAR. The study was conducted in accordance to relevant guidelines and regulations.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author credit statement

Waseem feeroze and Mohd younus shah wrote the manuscript, data collection and result interpret. Gurjinder singh participated in the design of this study, kumud borole and yunis saleem done data collection and analysis, tariq azad reviewed the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable

request.

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REFERENCES

1. D.X. Liu, J.Q. Liang, T.S. Fung, Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae), Encyclopedia of Virology (2) (2021) 428–40. doi: 10.1016/B978-0-12-809633-8.21501-X. Epub 2021 Mar 1. PMID: PMC7204879, <https://doi.org/10.1016/B978-0-12-809633-8.21501-X>
2. https://commons.wikimedia.org/wiki/File:3D_medical_animation_coronavirus_structure.jpg.a
3. P.C. Woo, Y. Huang, S.K. Lau, K.Y. Yuen, Coronavirus genomics and bioinformatics analysis. *Viruses* (8) (2010) 1804–20, <https://doi.org/10.3390/v2081803>
4. N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, T. Yu, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, *Lancet* 395 (10223) (2020) 507–513, [https://doi.org/10.1016/S0140-6736\(20\)30211-7](https://doi.org/10.1016/S0140-6736(20)30211-7)
5. <https://www.gavi.org/vaccineswork/covid-19-vaccine-race>.
6. H. Lu, C.W. Stratton, Y.W. Tang, The Wuhan SARS-CoV-2—What's next for China. *Journal of medical virology* (6) (2020) 546–7, <https://doi.org/10.1002/jmv.25738>
7. J.S. Peiris, S.T. Lai, L.L. Poon, Y. Guan, L.Y. Yam, W. Lim, J. Nicholls, W.K. Yee, W.W. Yan, M.T. Cheung, V.C.C. Cheng, K.H. Chan, D.N.C. Tsang, R.W.H. Yung, T.K. Ng, K.Y. Yuen, Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* (361) (2003) 1319–1325, [https://doi.org/10.1016/s0140-6736\(03\)13077-2](https://doi.org/10.1016/s0140-6736(03)13077-2)
8. Z. Xu, L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, S. Liu, P. Zhao, H. Liu, L. Zhu, Pathological findings of COVID-19 associated with acute respiratory distress syndrome, *Lancet Respiratory Med.* 8 (4) (2020) 420–422, [https://doi.org/10.1016/S2213-2600\(20\)30076-X](https://doi.org/10.1016/S2213-2600(20)30076-X)
9. L. Xu, J. Liu, M. Lu, D. Yang, X. Zheng, Liver injury during highly pathogenic human coronavirus infections. *Liver Int.* (40) (2020) 998–1004, <https://doi.org/10.1111/liv.14435>
10. P. Liu, L. Shi, W. Zhang, J. He, C. Liu, C. Zhao, S.K. Kong, J.F. Loo, D. Gu, L. Hu, Prevalence and genetic diversity analysis of human coronaviruses among cross-border children. *Virology journal* (1) (2017) 1–8, <https://doi.org/10.1186/s12985-017-0896-0>.

11. W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, L. Liu, H. Shan, C.L. Lei, D.S.C. Hui, B. Du, L.J. Li, G. Zeng, K.Y. Yuen, R.C. Chen, C.L. Tang, T. Wang, P.Y. Chen, J. Xiang, S.Y. Li, J.L. Wang, Z.J. Liang, Y.X. Peng, L. Wei, Y. Liu, Y.H. Hu, P. Peng, J.M. Wang, J.Y. Liu, Z. Chen, G. Li, Z.J. Zheng, S.Q. Qiu, J. Luo, C.J. Ye, S.Y. Zhu, N.S. Zhong, Clinical Characteristics of coronavirus Disease 2019 in China. *N Engl J Med* (382) (2020) 1708-1720, <https://doi.org/10.1056/NEJMoa2002032>.
12. M. Yang, M.H. Ng, C.K. Li, Thrombocytopenia in patients with severe acute respiratory syndrome. *Hematology* 10 (2) (2005) 101-5, <https://doi.org/10.1080/10245330400026170>.
13. P. Jolicoeur, L. Lamontagne, Impairment of bone marrow pre-B and B cells in MHV3 chronically-infected mice. In *Corona-and Related Viruses Adv. Exp. Med. Biol.* 380 (1995) 193-195, https://doi.org/10.1007/978-1-4615-1899-0_33.
14. G. Lippi, M. Plebani, B.M. Henry, Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. *Clin Chim Acta.* (506) (2020) 145-148, <https://doi.org/10.1016/j.cca.2020.03.022>
15. T.S. Ho, S.M. Wang, C.C. Liu, Historical review of pandemic influenza A in Taiwan, 2009. *Pediatr Neonatol* (51) (2010) 83-88, [https://doi.org/10.1016/S1875-9572\(10\)60016-2](https://doi.org/10.1016/S1875-9572(10)60016-2)
16. C.T. Wu, S.H. Hsia, J.L. Huang, Influenza B-associated rhabdomyolysis in Taiwanese children. *Acta Paediatr* (99) 2010 1701-1704, <https://doi.org/10.1111/j.1651-2227.2009.01595.x>
17. P. Jolicoeur, L. Lamontagne, Impaired T and B cell subpopulations involved in a chronic disease induced by mouse hepatitis virus type 3. *J Immunol* (153) (1994) 1318-1317. <https://doi.org/10.1128/jvi.69.10.6541-6547.1995>
18. M. Yang, C.K. Li, K. Li, K.L. Hon, M.H. Ng, P.K. Chan, T.F. Fok, Hematological findings in SARS patients and possible mechanisms. *International journal of molecular medicine* 14 (2) (2004) 311-5, <https://doi.org/10.3892/ijmm.14.2.311>
19. T. Li, Z. Qiu, Y. Han, Z. Wang, H. Fan, W. Lu, J. Xie, X. Ma, A. Wang, Rapid loss of both CD4+ and CD8+ T lymphocyte subsets during the acute phase of severe acute respiratory syndrome. *Chin Med J (Engl)* 116 (7) (2003) 985-7, PMID: 12890367
20. P.P. Bloom, E.A. Meyerowitz, Z. Reinus, M. Daidone, J. Gustafson, A.Y. Kim, E. Schaefer, R.T. Chung, Liver biochemistries in hospitalized patients with COVID-19. *Hepatology* 73(3) (2021) 890-900, <https://doi.org/10.1002/hep.31326>
21. Q. Cai, D. Huang, H. Yu, Z. Zhu, Z. Xia, Y. Su, Z. Li, G. Zhou, J. Gou, J. Qu, Y. Sun, COVID-19: Abnormal liver function tests. *Journal of hepatology* 73(3) (2020) 566-74, <https://doi.org/10.1016/j.jhep.2020.04.006>
22. C. Zhang, L. Shi, F.S. Wang, Liver injury in COVID-19: management and challenges. *The lancet Gastroenterology & hepatology* 5(5) (2020) 428-30, [https://doi.org/10.1016/S2468-1253\(20\)30057-1](https://doi.org/10.1016/S2468-1253(20)30057-1)
23. G. MacLaren, D. Fisher, D. Brodie, Preparing for the most critically ill patients with COVID-19: the potential role of extracorporeal membrane oxygenation. *JAMA* 323(13) (2020) 1245-6, <https://doi.org/10.1001/jama.2020.2342>
24. W. Zhang, Imaging changes of severe COVID-19 pneumonia in advanced stage. *Intensive care medicine* 46(5) (2020) 841-3, <https://doi.org/10.1007/s00134-020-05990-y>
25. N. Doria-Rose, M.S. Suthar, M. Makowski, S. O'Connell, A.B. McDermott, B. Flach, J.E. Ledgerwood, J.R. Mascola, B.S. Graham, B.C. Lin, S. O'Dell, S.D. Schmidt, A.T. Widge, V.V. Edara, E.J. Anderson, L. Lai, K. Floyd, N.G. Roupheal, V. Zarnitsyna, P.C. Roberts, M. Makhene, W. Buchanan, C.J. Luke, J.H. Beigel, L.A. Jackson, K.M. Neuzil, H. Bennett, B. Leav, J. Albert, P. Kunwar, mRNA-1273 Study Group. Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid19. *N Engl J Med* 384(23) (2021) 2259-226, <https://doi.org/10.1056/NEJMc2103916>
26. W.J. Seo, J. Kang, H.K. Kang, S.H. Park, H.K. Koo, H.K. Park, S.S. Lee, J.E. Song, Y.G. Kwak, J. Kang, Impact of prior vaccination on clinical outcomes of patients with COVID-19. *Emerging Microbes & Infections.* 11(1) (2022) 1316-24. <https://doi.org/10.1080/22221751.2022.2069516>

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