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Clinical Significance of Neutralizing Antibodies in COVID-19: Implications for Disease Prognosis

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Abstract: The pathogenesis of COVID-19 highlights a complex relationship between disease severity and neutralizing antibodies (NAbs). We aimed to investigate the relationship between total NAb (tNAb) levels, presence of potential neutralization inhibiting antibodies (pNAbs), and the clinical outcomes of COVID-19 patients. Patients aged ≥ 18 years diagnosed between October 2021 and September 2022 were grouped by symptom severity. Blood samples were taken at two time points and data on demographics, epidemiology, and vaccination were recorded. The tNAbs and pNAbs were measured by enhanced chemiluminescence assay and surrogate virus neutralization test, respectively. Among 68 tNAbs and 52 pNAbs results, 26 (38.2%) patients had severe infection. The 28-day mortality rate was 16.2% (n=11). The tNAbs levels in the control blood samples (CBS) were significantly higher than those of the admission blood samples (ABS) ($p < 0.001$). The statistical analysis showed no relation between disease severity and tNAb titers. Vaccinated patients had significantly higher tNAbs in ABS and CBS ($p < 0.001$ and $p < 0.001$, respectively). Presence of pNAbs in the ABS correlated with lower 28-day mortality ($p = 0.026$) and milder disease course ($p = 0.041$). Although these findings support a correlation between tNAbs and disease severity and mortality, this finding seems to be related to their formation rather than their effect on the clinical outcomes.

Keywords: neutralizing antibodies; neutralization inhibition; COVID-19 severity; pathogenesis; mortality; vaccination

1. Introduction

More than five years have gone by since the emergence of the COVID-19 pandemic. Although a substantial body of research has been conducted on various aspects of the disease during this time, there are still major gaps in our understanding of the disease.

The initial stage of viral infection occurs when the receptor binding domain (RBD) of the spike (S) protein of the virus binds to the angiotensin converting enzyme 2 (ACE2) receptor on the host cell. Following the entry, cellular pathogen receptors recognize the virus, and the immune system is activated. Antiviral response involves activation of the INF I&III and downstream signaling pathways. It has been shown that in severely ill patients, these antiviral signaling pathways are interfered with by the viral structural and non-structural products. Post intracellular replication, the virus is shed and antibody response ensues [1,2].

RBD is the main site of action of neutralizing antibodies (NAbs) [3,4]. The affinity and titers of the NAbs determine their efficacy [5–8]. However, in the context of Covid-19 disease, elevated serum NAbs titers are not correlated with protective immunity in many cases [9–11]. Although numerous studies demonstrated the efficacy of potent NAbs in viral neutralization assays, they fail to explain serious illness and / or fatality in their presence at elevated titers [11,12]. In addition, the virus can evade NAbs by mutations. Despite this, neutralization activity is not

completely lost in previously infected, vaccinated, or hybrid immune individuals thanks to broadly neutralising antibody response [1].

Several studies demonstrated that the disease progresses more severely in patients with low NAbs and is milder in those with high NAbs [13,14]. It has also been observed that humoral immunity is more robust and NAb levels are elevated in individuals exhibiting severe disease symptoms [15]. In other words, the patient's clinical condition determines the type of the antibody response. It was postulated that the neutralization efficacy of anti-RBD NAbs is heterogeneous, with some being ineffective and hence the discrepancies on the role of high titer NAbs in protection and recovery from the disease [3,5].

The anti-SARS CoV-2 vaccines were developed and applied after the pandemic largely touched the most vulnerable people in the population. Even after then, the efficacy of the vaccines provided conflicting outcomes. It has been shown that the antibody response formed through infection or after vaccination is inversely correlated to the severity of the disease [16]. Moreover, the vaccine effectiveness is better against symptomatic infection than asymptomatic infection [17]. However, it has been demonstrated that the NAbs formed after vaccination are not a marker for protection from the disease [18]. The vast majority of people who fail to become ill post-viral exposure and that those who become mildly ill lack NAbs

At the conception of this project, the role of NAbs of all immunoglobulin classes (tNAbs) in the pathogenesis and/or protection was unclear. Therefore, we set out to investigate the correlation, if any, of tNAbs with the clinical course of the disease as well as other hematological and biochemical parameters. The neutralisation inhibitory properties of the tNAbs were also investigated.

2. Materials and Methods

2.1. Study Population

Patients aged ≥ 18 years admitted to the pandemic wards of Recep Tayyip Erdoğan University Research Hospital between 01.10.2021 and 01.09.2022 were included in the study. Covid-19 diagnosis was confirmed by SARS-CoV-2 real time polymerase chain reaction (PCR). Clinical group allocation of patients was made as follows: Patients without symptoms were defined as asymptomatic, those with mild symptoms but no pneumonia or requirement for oxygenation were defined as mildly symptomatic, patients with pneumonia or needing oxygenation were defined as moderately symptomatic, and patients who needed follow-up in the intensive care unit were defined as severely symptomatic. Patients vaccinated with four doses of any COVID-19 vaccine were considered to have received a full dose vaccination. Epidemiological data, demographic characteristics, and vaccination information against COVID-19 were recorded.

2.2. Blood Samples

Blood samples were collected from patients as follows. The admission blood samples (ABS) were collected within the first two weeks after the detection of PCR positivity, and the control blood samples (CBS) were collected at least one week after the ABS and stored at -80°C .

2.3. Serologic Assay

2.3.1. IgM and IgG Measurements

IgG and IgM anti SARS-CoV-2 RBD antibodies in ABS and CBS were qualitatively measured by lateral diffusion using the COVID-19 IgG/IgM Rapid Test kit (Meril®, Cat No: NCVRPD-02, India) by following the manufacturer's instructions.

2.3.2. Neutralization Antibody Measurements

The Elecsys (Roche®) Anti-SARS-CoV-2 S immunoassay kit was used for quantitative in vitro determination of antibodies against the RBD of the SARS-CoV-2 S protein. The assay employs a recombinant form of the RBD of the S antigen to detect all classes of antibodies in a double-antigen sandwich method. Results are reported in units/mL and defined as total neutralizing antibody levels (tNABs). The kit was used in accordance with the manufacturer's instructions.

In the second part of the study, the blood samples of patients were used for neutralization inhibition test. In this test, Elabscience (Genscript®) SARS-CoV-2 Surrogate Virus Neutralization Kit was used and the results were obtained qualitatively. This test is based on competitive ELISA in which tNABs compete for binding to solid phase-immobilised ACE2 with RBD-horseradish peroxidase conjugate. The assumption is that among the tNABs that bind to RBD, only those with the ACE2 receptor binding potential (pNABs) will yield positive result. The manufacturer states that this method has a specificity of 99.93% and a sensitivity of 95–100%.

2.4. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp; 2013). The normality of variables was examined using Kolmogorov-Smirnov/Shapiro-Wilk tests. Median values, Mann-Whitney U, Wilcoxon and Kruskal Wallis tests were used for variables not conforming to normal distribution. Chi-square and Mc Namar tests were used to compare the difference between the groups.

Correlation analyses were applied to determine the strength and direction of the linear relationship between two variables. Since the data were not normally distributed, the Spearman rank correlation was used to evaluate the ordinal relationship.

Multinomial logistic regression analysis was performed for multi-category dependent variables. In this analysis, the effect of independent variables on each category was evaluated and odds ratios were calculated on a category basis. Logistic regression was used for binary dependent variables. In this model, the effect of independent variables on mortality was evaluated and the significance of independent variables was interpreted using the Wald test and odds ratios (Exp(B)). A p value of ≤ 0.05 was considered statistically significant for all types of analysis.

3. Results

3.1. Demographic Characteristics

In total, the tNABs of 68 and the pNABs of 52 patients were analysed. Of the patients, 35 (51.5%) were female, the median age was 67 years (range, 25-94 years). Fourteen (20.6%) patients were asymptomatic or had mild infection, 28 (41.2%) had moderate infection, and 26 (38.2%) had severe infection. Forty-one (60.3%) patients had at least one comorbidity, the most common being cardiovascular disease (n=27, 39.7%) followed by essential hypertension (n=25, 36.8%). The median hospital stay was 17 days (range 1 to 112 days), 21 (30.9%) patients died, and the 28-day mortality rate was 16.2% (n=11).

3.2. IgM and IgG results

COVID-19 anti-spike IgM was detected in 22 (32.4%) of the ABS and 64.7% (n=44) of the CBS. Similarly, COVID-19 anti-spike IgG was detected in 37 (54.4%) of the ABS and 94.1% (n=64) of the CBS.

3.3 Neutralization Antibody Results

The tNABs levels in the CBS (median 18140 U/ml, range 0.4 to 927920) were found to be statistically significantly higher than those in the ABS (median 488.2 U/ml, range 0.4 to 571870) ($p < 0.001$). While pNABs were present in 41 (78.8%) of the patients in ABS, this number was found to be 47 (90.4%) in CBS and no statistically significant difference was detected ($p = 0.146$).

On the other hand, the tNAb levels in the ABS were found to be statistically significantly higher in the COVID-19 vaccinees compared to those who were not, and in patients positive for IgM or IgG compared to the negatives ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). This implies that if present, IgA anti-RBD had no demonstrable contribution to RBD binding (neutralisation). The tNAbs levels of CBS was statistically significantly higher in patients with hypertension than those without, and in COVID-19 vaccinated ones than those without ($p = 0.022$ and $p < 0.001$, respectively). No significant relationship was found between the other investigated parameters and tNAbs levels ($p > 0.05$, Table 1).

Table 1. Comparison of epidemiological data with patients' total neutralizing antibody levels

Parameter		Admission (n=68) Median (min-max)		P value	Control (n=68) Median (min-max)	P value
Gender	Female	477.7 (0.4 – 343370)		0.854	11080 (0.4-927920)	0.902
	Male	498.7 (0.4 – 571870)			32050 (0.4-474800)	
Disease severity	Asymptomatic - Mild	2441 (0.4 – 98660)		0.496	16260 (32.49-474800)	0.699
	Moderate	1027.35 (0.4 – 571870)			67360 (0.4-927920)	
	Severe	211.75 (0.4 – 73640)			17330 (0.51-706590)	
Comorbidities	Hypertension	No	144.7 (0.4 – 571870)	0.072	1856 (0.4-474800)	0.022
		Yes	3555 (0.4 – 343370)		71530 (11.13-927920)	
	Diabetes Mellitus	No	447.75 (0.4 – 293500)	0.326	12875 (0.4-927920)	0.062
		Yes	2026.8 (0.4 – 571870)		71985 (27.7-706590)	
	Chronic Kidney Disease	No	447.7 (0.4 – 571870)	0.402	15450 (0.4-927920)	0.962
		Yes	588.3 (16.8 – 90260)		27070 (29-139660)	
	Cardiovascular Disease	No	299 (0.4 – 571870)	0.400	8088 (0.4-474800)	0.290
		Yes	1022 (0.4 – 122080)		44240 (4-927920)	
	Immunosuppression	No	588.3 (0.4 – 343370)	0.965	18110 (0.4-927920)	0.702
		Yes	178.8 (0.4 – 571870)		30265 (0.4-328710)	

Chronic Obstructive Pulmonary Disease	No	477.7 (0.4 – 0.482	19990 (0.4-927920)	0.429
	Yes	571870)	14670 (4-60440)	
COVID-19 Vaccination	No	5.33 (0.4-1619)	<0.001	126.8 (0.4-126360)
	Yes	5610 (0.4-571870)		66480 (0.4-927920)
Full-dose COVID-19 vaccination (n=49) ¹	No	4451 (0.4 – 0.585	72440 (0.4-927920)	0.157
	Yes	571870)	24170 (29-170410)	
Time since LV ² (n=49)	<6 months	6682.5 (0.4- 0.758	71985 (0.4-927920)	0.743
	>6 months	343370)	46110 (37.7-393440)	
COVID-19 IgM ABS ³	Negative	211.75 (0.4 – <0.001		
	Positive	66090)		
COVID-19 IgM CBS ⁴	Negative	69600 (5.33 –	15450 (5.07-313170)	0.488
	Positive	571870)	38145 (0.4-927920)	
COVID-19 IgG ABS ³	Negative	58.1 (0.4 – 66090)	<0.001	
	Positive	14210 (0.4 - 571870		
COVID-19 IgG CBS ⁴	Negative		208.09 (5.07-16290)	0.090
	Positive		27070 (0.4-927920)	
28 day mortality	No	677.9 (0.4 – 0.874	16230 (0.4-927920)	0.659
	Yes	571870)	44240 (29-706590)	
pNAbs ABS ³	No	42.9 (17-343370)	0.148	
	Yes	5610 (4-571870)		
pNAbs CBS ⁴	No		117230 (22090- 0.167	
	Yes		706590)	
			62600 (4-927920)	

¹Only vaccinated people were examined, ²Last vaccination, ³Admission blood samples, ⁴Control blood samples.

The 28-day mortality was statistically significantly lower in patients with pNAbs in both ABS and CBS compared to those without (p=0.025 and p=0.043, respectively). No significant relationship was found between the other compared parameters and pNAbs levels (p>0.05, Table 2).

Table 2. Comparison of epidemiological and laboratory data with patients' potent neutralizing antibody status.

Parameter		Admission (n=52)		p	Control (n=52)		p
		No	Yes		No	Yes	
Cinsiyet (n [%])	Kadın	8 (30.8)	18 (69.2)	0.174	3 (11.5)	23 (88.5)	1.000
	Erkek	3 (11.5)	23 (88.5)		2 (7.7)	24 (92.3)	
Age (Year) (median [min-max])		67 (62 – 85)	67 (28 – 89)	0.419	77 (65- 89)	67 (28- 88)	0.232
Hospital stay (median [min-max])		21 (8 – 83)	15 (1 – 85)	0.158	21 (14- 23)	18 (1- 85)	0.664
Disease severity (n [%])	Asymptomatic - Mild	0 (0)	11 (100)	0.089	1 (9.1)	10 (90.9)	0.600
	Moderate	4 (20)	16 (80)		1 (5)	19 (95)	
	Severe	7 (33.3)	14 (66.7)		3 (14.3)	18 (85.7)	
Comorbidities (n [%])	Hypertension	No	5 (16.7)	0.495	1 (3.3)	29 (96.7)	0.149
		Yes	6 (27.3)		16 (72.7)	4 (18.2)	
	Diabetes Mellitus	No	6 (17.1)	0.470	3 (8.6)	32 (91.4)	1.000
		Yes	5 (29.4)		12 (70.6)	2 (11.8)	
	Chronic Kidney Disease	No	8 (20)	0.701	3 (7.5)	37 (92.5)	0.325
		Yes	3 (25)		9 (75)	2 (16.7)	
	Cardiovascular Disease	No	4 (14.3)	0.332	1 (3.6)	27 (96.4)	0.169
		Yes	7 (29.2)		17 (70.8)	4 (16.7)	
	Immunosuppression	No	9 (21.4)	1.000	5 (11.9)	37 (88.1)	0.569
		Yes	2 (20)		8 (80)	0 (0)	
	Chronic Obstructive Pulmonary Disease	No	11 (22.4)	1.000	5 (10.2)	44 (89.8)	1.000
		Yes	0 (0)		3 (100)	0 (0)	
		No	1 (14.3)	1.000	0 (0)	7 (100)	1.000

COVID-19 Vaccination (n [%])	Yes	10 (22.2)	35 (77.8)		5 (11.1)	40 (88.9)	
Full-dose COVID-19 vaccination ¹ (n=45) [%]	No	8 (22.9)	27 (77.1)	1.000	4 (11.4)	31 (88.6)	1.000
	Yes	2 (20)	8 (80)		1 (10)	9 (90)	
Time since LV ² (n=45)	<6 months	7 (25.9)	20 (74.1)	0.716	4 (14.8)	23 (85.2)	0.634
	>6 months	3 (16.7)	15 (83.3)		1 (5.6)	17 (94.4)	
COVID-19 IgM ABS ³ (n [%])	Negative	8 (25.8)	23 (74.2)	0.491			
	Positive	3 (14.3)	18 (85.7)				
COVID-19 IgM CBS ⁴ (n [%])	Negative				0 (0)	18 (100)	0.150
	Positive				5 (14.7)	29 (85.3)	
COVID-19 IgG ABS ³ (n [%])	Negative	7 (36.8)	12 (63.2)	0.074			
	Positive	4 (12.1)	29 (87.9)				
COVID-19 IgG CBS ⁴ (n [%])					0 (0)	2 (100)	1.000
					5 (10)	45 (90)	
28 day mortality (n [%])	No	6 (14.3)	36 (85.7)	0.025	2 (4.8)	40 (95.2)	0.043
	Yes	5 (50)	5 (50)		3 (30)	7 (70)	

¹Only vaccinated people were examined, ²Last vaccination, ³Admission blood samples, ⁴Control blood samples.

3.4. Vaccination Results

Forty-nine (72%) of the patients received two and 10 (14.7%) received 4 doses of Covid-19 vaccine regardless of type. The vaccination rate with the inactivated vaccine (Sinovac®) among all patients was 64.7% (n=44), while the mRNA vaccine (BioNTech®) was 22.1% (n=15). The median time between SARS-CoV-2 PCR positivity and the last vaccination date of 49 vaccinees was 155 days (range 3 to 362). Of the vaccinated patients, 20 (40.8%) had severe infection, 19 (38.8) had moderate infection, and 10 (20.4%) had asymptomatic-mild infection, and the 28-day mortality rate was 22.4% (n=11).

3.5. Correlation Analysis Results

Spearman correlation analysis results revealed significant relationships among various variables. A negative correlation was found between the presence of pNAbs in ABS and CBS and 28-day mortality ($r=-0.345$, $p=0.012$ and $r=-0.337$, $p=0.014$, respectively). Negative correlations were also found between full-dose vaccine status and 28-day mortality ($r=-0.331$, $p=0.002$). No correlation was found between pNAbs and tNAbs in CBS ($r=0.193$, $p=0.169$) or in ABS ($r=0.202$, $p=0.150$). Further, there was no correlation between pNAbs in ABS and CBS ($r=0.151$, $p=0.287$). However, there was a positive correlation between tNAbs in ABS and CBS ($r=0.763$, $p=0.000$).

3.6. Regression Analysis Results

According to the results of multinomial logistic regression analysis used to evaluate independent risk factors thought to be associated with the severity of the disease in patients

hospitalized with COVID-19, the probability of the disease being severely symptomatic was 538 times more than the probability of being asymptomatic or mildly symptomatic in IgG positive patients in their blood at admission (OR 538.7; CI 1.175-247056.7; p= 0.044). In patients with pNAbs on admission, the probability of the disease being asymptomatic or mildly symptomatic was 0.872 times more (1.14 times less) than the probability of being severely symptomatic (OR 0.872; CI 0.772-0.984; p= 0.026, Table 3).

Table 3. Results of multinomial logistic regression analysis in evaluating independent risk factors affecting the severity of COVID-19.

Risk Factors		Multinomial analyse				
		95% Confidence Interval				
		for Exp(B)				
		B	Odd's ratio	Lower Bound	Upper Bound	p value
Moderate symptomatic	IgM in ABS ¹	-3.521	0.03	0.001	1.352	0.071
	IgG in ABS ¹	5.213	183.6	0.546	61699.735	0.079
	Days since LV ²	0.006	1.006	0.992	1.019	0.419
	tNAbs in ABS ¹	0.000	1.000	1.000	1.000	0.184
	tNAbs in CBS ³	0.000	1.000	1.000	1.000	0.240
	pNAbs in ABS ¹	-0.098	0.907	0.806	1.021	0.107
	pNAbs in CBS ³	0.040	1.041	0.983	1.102	0.174
Severe symptomatic	IgM in ABS ¹	-0.485	0.616	0.018	30.948	0.787
	IgG in ABS ¹	6.289	538.7	1.175	247056.786	0.044
	Days since LV ¹	0.007	1.007	0.993	1.021	0.350
	tNAbs in ABS ¹	0.000	1.000	1.000	1.000	0.708
	tNAbs in CBS ³	0.000	1.000	1.000	1.000	0.310
	pNAbs in ABS ¹	-0.137	0.872	0.772	0.984	0.026
	pNAbs in CBS ³	0.021	1.021	0.974	1.071	0.388

¹Admission blood samples, ²last vaccination, ³control blood samples.

According to the results of logistic regression analysis used to evaluate independent risk factors thought to be associated with 28-day mortality in patients, the presence of pNAbs on the day of admission was shown to be associated with lower mortality by 0.914 times (OR 0.914; p=0.041). No statistical relationship was shown between other parameters thought to add significance to the model and 28-day mortality (Table 4).

Table 4. Results of logistic regression analysis in evaluating independent risk factors affecting 28-day mortality in patients

Risk factors	Logistic analysis		
	B	Odd's ratio	p value
IgM in ABS ¹	-2.501	0.082	0.402
IgM in CBS ²	-0.175	0.840	0.915

IgG in ABS ¹	-3.491	0.03	0.341
IgG in CBS ²	-15.590	0.000	1.000
Days since LV ³	-0.013	0.987	0.161
tNAbs in ABS ¹	0.000	1.000	0.141
tNAbs in CBS ²	0.000	1.000	0.301
pNAbs in ABS ¹	-0.089	0.914	0.041
pNAbs in CBS	-0.004	0.006	0.792

¹ Admission blood samples, ² Control blood samples, ³last vaccination.

4. Discussion

In this study, patients with pNAbs in ABS were 0.872 times more likely to be asymptomatic or mildly symptomatic (or 1.14 times less likely to be severely symptomatic) compared to being severely symptomatic and the 28-day mortality was lower than those without pNAbs. The tNAbs values were found to be high in both ABS and CBS in vaccinated patients.

No relationship was found between tNAbs measurements and clinical severity. However, multinomial regression analysis revealed that in patients with pNAbs in ABS, the probability of being asymptomatic or mildly symptomatic was greater than the probability of being severely symptomatic. A similar relationship was not observed for pNAbs in CBS. In a review by Mink S. et al., it was stated that high antibody levels reduce the risk of infection and disease severity [19]. Similarly, Monroe JM. et al. emphasized in a study that tNAbs are protective against SAR-COV-2 infection and symptomatic disease development [20]. In our study, the pNAbs in ABS support these findings. However, our results do not support these studies in terms of tNAbs. Takeshita M. et al. reported no tNAbs formation in asymptomatic / mildly ill patients [13]. Cavlek et al. noted age-related variability in tNAbs titers [21]. Chen W. et al. have found that tNAbs may be undetectable in the blood after recovery in some patients [22], which was also the case in this study. Xu J. et al., in a review, stated that the antibody response in severe COVID-19 patients was higher than in asymptomatic patients, but no difference is observed in the early stages of the disease [23]. Shrivasta S et al. showed that tNAbs merely indicates symptomatic infection but seems unrelated to protection and recovery [14]. Combining our findings with these diverse literature suggests that disease severity cannot be solely attributed to the presence or titers of tNAbs and other contributing factors must be considered (e.g. inadequate innate immune response). These variable results indicate that the clinical condition of the patient will determine the type of the tNAbs results [24]. The more severe the disease the higher the tNAbs titers, with rare exceptions. In the very few exceptional cases, patients recover or pass without any detectable anti-viral antibodies, of which there were two in this study. This hypothesis is further supported by the fact that the neutralizing efficacy of tNAbs is not the sole determining function for antiviral [6,8].

When evaluated in terms of mortality, our study showed that 28-day mortality was lower in patients with pNAbs in ABS, while there was no significant difference in tNAbs measurements. Numerous studies have shown that higher antibody titers are associated with lower mortality rates [19,25,26]. Our findings regarding pNAbs are consistent with these studies.

As expected, the tNAbs titres in CBS were statistically significantly higher than those of ABS. However, no relationship was found between the tNAbs of either ABS or CBS and the presence of pNAbs. There was no correlation between tNAbs and pNAbs. The tNAbs and pNAbs results in our study were not interrelated implying that not all tNAbs in our study had pNAb activity.

Another important parameter examined in our study was the relationship between tNAbs and vaccination. Previous publications showed that tNAbs levels peak approximately 14 days after vaccination, but only 50% of them can be detected in the blood [8]. Additionally, it has been reported that the tNAbs levels in some patients decline to undetectable levels in the blood within six months after the second dose of vaccination [27]. In our patients, no relationship was found between the time

of the last vaccination and the tNAb titers in vaccinated patients. Studies in the literature summarize that antibody levels against SARS-CoV-2 are low in the naive and unvaccinated groups, but these rates reach 100% after the second dose [8]. In our study, all vaccinated patients received at least two doses of vaccine at least 14 days prior to sampling except one individual. In addition, it was observed that tNAbs in ABS and CBS of vaccinated patients were significantly higher compared to unvaccinated patients. This is in line with the literature. On the other hand, no relationship was shown between the presence of pNAbs and vaccination status in our study. These contradictory findings may be due to the relatively low sample size.

It is known that when patients with hypertension have COVID-19, the disease progresses more severely [28]. Also, studies have shown that COVID-19 significantly affects the cardiovascular system and one of its most common complications is the emergence of HT [29]. In our patients, tNAb titers in CBS were significantly higher in HT patients than in those without. The reason for this might be that the patients with HT have renin-angiotensin system abnormalities and are more prone to severe infection and antibody responses in these individuals may be variable [30].

In this study, the number of participants in the different clinical categories of COVID-19 turned out to be relatively small. One of the reasons for this is that the study was carried out during the mid-to-late stages of the pandemic, and the other is the failure of some of the recruits to come to hospital for donating the control blood. This limited or prevented obtaining statistically significant results for some parameters and created limitations in terms of the generalizability of the findings. Although the vast majority of patients were vaccinated, the great variety in vaccine types and doses prevented some sub-analyses.

5. Conclusions

In this study, as in many others, it is evident that anti-SARS CoV-2 neutralizing antibody generation is correlated with disease severity and mortality, although this was not consistent in all cases. It is also supported that all clinical outcomes cannot be attributed solely to the presence or levels of total neutralizing antibodies. Overall, susceptibility to infection and severe viral illness must be determined by the innate immune system.

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Data Availability Statement: Original data supporting the findings of this study are available.

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