

# comparison neutralizing Ab

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**COMPARISON OF NEUTRALIZING SARS COV-2 IGG ANTIBODY LEVELS  
IN HOSPITALIZED PATIENTS AT DR. MOHAMMAD HOESIN PALEMBANG  
HOSPITAL**

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**ABSTRACT**

COVID-19 is an acute respiratory disease that spreads rapidly throughout the world caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Published research on levels of SARS-CoV-2 antibodies in hospitalized COVID-19 patients is still limited. Research on sero-epidemiology and its correlation with the clinical severity of COVID-19 is needed as a database for pandemic governance, especially research on neutralizing antibodies. This study was designed to compare the levels of IgG SARS-CoV-2 on the tenth day in hospitalized patients with various clinical severity at RSUP Dr. Mohammad Hoesin Palembang. This research is an observational-analytic study with a cross-sectional design. The study was conducted in the COVID-19 isolation ward of RSMH Palembang from December 2021 to March 2022. IgG SARS-CoV-2 level was measured in patients with confirmed COVID-19. Differences in IgG values of SARS-CoV-2 were tested with the Mann Whitney test. The effect of various confounding variables in this study was tested using bivariate and multivariate tests in the form of a chi-square test and multiple linear regression on the scale of the dependent variable. The study included 50 patients, consisting of 22 patients with mild-moderate severity and 28 patients with severely-critically ill. Bivariate analysis showed there was no significant difference of IgG SARS-CoV-2 between two groups. From the data analysis using the ROC curve, the cut-off point for SARS-CoV-2 IgG levels in predicting the severity of COVID-19 disease was 500 AU/mL. IgG SARS-CoV-2 level of more than 500 AU/mL causes an increased risk of 3.69 (95% CI 1.13-11.98) to suffer from severe-critical COVID-19. Serum IgG SARS-CoV-2 levels were found to be significantly higher in patients with severe-critical symptoms than patients with mild-moderate symptoms in COVID-19 hospitalized patients at Dr. Mohammad Hoesin Palembang hospital.

**Keywords:** COVID-19, severity of COVID-19, IgG SARS-CoV-2

## INTRODUCTION

*Coronavirus Disease*(COVID-19) is an infectious disease that causes acute respiratory syndrome caused by Coronavirus 2 (SARS-CoV-2). The disease was first documented in humans in China in December 2019 and was declared a global pandemic by the World Health Organization (WHO) in March 2020. The ongoing SARS-CoV-2 pandemic has resulted in 173,005,553 clinically confirmed cases of COVID-19 and causes the death of 2.15% of cases or 3,727,605 lives worldwide (as of 7 June 2021).

On the same date, Indonesia has reported 1,856,038 confirmed cases of COVID-19 with a mortality rate of 51,612 cases or 2.78% of cases. In South Sumatra, 25,339 confirmed cases of COVID-19 were reported with a mortality rate of 5.08% or around 1289 cases of death.

This data shows that South Sumatra is one of the provinces with the highest COVID-19 mortality rate, although it can also show positive case findings have not been maximized. This was reported by Wirawan et al who showed that the gap in the availability of health services before the COVID-19 pandemic correlated with the incidence and reports of the level of death of COVID-19 in Indonesia. The absence of diagnostic facilities in several provinces is reported to have caused confirmed case reports to be lower than it should have been and to have resulted in a small number of confirmed case reports.

The SARS-CoV-2 RNA genome encodes 29 structural and nonstructural proteins, including spike protein (S), envelope protein (E), membrane protein (M), nucleocapsid protein (N), and ORF1a/b polyprotein. Glycoprotein S plays a role in attachment and

facilitates entry of viruses into various host cells through binding mediated by binding to the angiotensin-converting-enzyme-2 (ACE-2) receptor.

Virus-specific neutralizing antibodies (nAbs) play an important role in reducing viral replication and increasing the body's efforts to clear viruses in the healing process and efforts to prevent reinfection. nAbs that have been reported to bind primarily to the receptor-binding-domain (RBD) of the SARS-CoV-2 S glycoprotein (spike-RBD), effectively blocking viral attachment and entry into host cells. Therefore, serological tests, especially those that detect nAbs, are essential to determine the onset of a humoral immune response. Measurement of SARS-CoV-2 anti-glycoprotein S antibodies can help determine specific antibody titers, facilitate longitudinal monitoring of antibody responses in individual patients, and specifically monitor antibody responses to viruses.

Recent studies have shown that IgM anti-spike-RBD is detectable on day 10 and forms rapidly by day 19 of symptoms, while IgG anti-spike-RBD begins to form in the second week (day eight to day ten), increasing in the third week or fourth, and persisting for more than six months. Another report showed that IgG and IgM seroconversion began to be detected on the tenth day.

The level of nAbs is reported to correlate with viral load and is therefore reported to be related to the severity of COVID-19. Studies in Hong Kong, China, and Spain have shown that serum IgG titers are related to disease severity.

In Indonesia, published research on the dynamics of SARS-CoV-2 antibody levels at various

levels of illness in inpatients with COVID-19 is still limited. Research on sero-epidemiology and its correlation with clinical severity of COVID-19 is needed as a database for pandemic management, especially research on neutralizing antibodies. This study was designed to compare SARS-CoV-2 IgG levels on the tenth day in inpatients with various levels of disease severity at the Internal Medicine Department of Dr. Mohammad Hoesin Palembang.

#### METHODS

This type of research is an observational analytic study with a cross-sectional design. The research was conducted at Dr. Mohammad Hoesin Palembang with a study population of COVID-19 patients treated at RSUP Dr. Mohammad Hoesin Palembang in December 2021 to March 2022.

Inclusion Criteria:

1. Individuals aged 18 to 59 years
2. Individuals with COVID-19 who are being treated at RSUP Dr. Mohammad Hoesin Palembang
3. Willing to participate in the research and sign informed consent

Exclusion Criteria

1. Patients with a history of autoimmune disorders and immunodeficiencies.
2. Patients with comorbid history of malignancy.
3. Patients who refused informed consent to participate in the study.
4. COVID-19 vaccination history.

The selection of research subjects was carried out using a selective proportional sampling technique, namely patients who met the inclusion criteria were taken as samples until they met the number set for each group. The patient was

taken at RSUP Dr. Mohammad Hoesin Palembang.

Subject characteristics were analyzed univariately and presented in the form of a frequency distribution table for categorical. Numericals are presented with mean  $\pm$  SD / median (minimum - maximum) depending on the distribution of normal or abnormal data. The data will be presented in the form of narratives, tables and graphs.

All numerical data were tested for distribution using the Shapiro-Wilk normality test. Numerical data that is normally distributed is presented using the mean  $\pm$  standard deviation and for data that is not normally distributed, the data is presented in median form (minimum value - maximum value). Differences in SARS-CoV-2 IgG values were tested with the Mann Whitney test. The influence of various confounding variables in this study was tested using bivariate and multivariate tests in the form of chi-square tests and multiple linear regression on the scale of the dependent variable. Based on the normality test for all numerical variables, it was found that age and IgG levels were not normally distributed. Descriptive analysis in this study is the processing of initial data on categorical variables (nominal/ordinal) and numerical variables (ratio/interval).

Research ethics were obtained from the Health Research Ethics Committee (KEPK) RSMH Palembang. The research sample was given an explanation of the research objectives and procedures related to the research and signed a consent form to participate in this research. The results obtained from this study were informed to the RSMH Palembang.

## RESULTS

### Description of Demographic Characteristics Data

The research variables included in the demographic characteristics of this study consisted of age, gender, history of hypertension, history of diabetes mellitus, and history of chronic kidney disease. The general characteristics of the study subjects including age, sex, and comorbidities are shown in Table 1. In this study, the age of the research subjects was obtained with a median value of 42 in the range of 20 to 59 years. There was no significant age difference between the mild-moderate and critical-severe groups.

Based on gender, the study subjects consisted of 25 (50%) males and 25 (50%) females. In the comparative analysis, it was found that the male sex was more common in the critical weight group ( $p < 0.01$ ). Meanwhile, the comorbidities reported were diabetes mellitus, hypertension, and chronic kidney disease. In the study subjects, 34% of subjects had comorbid diabetes mellitus, 28% had comorbid hypertension, and 8% were chronic kidney disease patients. There was no significant difference in the proportion of patients with comorbidities in the mild-moderate and severe-critical groups.

Table 1. Characteristics of Research Subjects

Variable	Research Subjects (n=50)	Mild-moderate (n=22)	Critical-Heavy (n=28)	P
Age (Years)	42 (20-59)	38.4±2.6	48.5 (22-58)	0.16 <sup>1</sup>
Gender				
Man	25 (50%)	6 (27.3%)	19 (67.9%)	<0.01 <sup>2</sup>
Woman	25 (50%)	16 (72.7%)	9 (32.1%)	
Comorbid				
DMT2	17 (34%)	8 (36.4%)	9 (32.1%)	0.95 <sup>1</sup>
Hypertension	14 (28%)	8 (36.4%)	6 (21.4%)	0.88 <sup>1</sup>
CKD	4 (8%)	2 (9.1%)	2 (7.1%)	0.76 <sup>1</sup>
Pregnancy and Puerperium	12 (24%)	6 (27.3%)	6 (21.4%)	0.88 <sup>1</sup>
Clinical Parameters				
Oxygen Saturation (%)	96 (30-99)	98 (94-99)	83.5 (30-99)	<0.01 <sup>1</sup>
Breath	26 (18-	23 (20-28)	28 (18-36)	<0.01 <sup>1</sup>

Rate (x/min)	36)			
Die	8(16%)	1 (4.5%)	7 (25%)	0.04 <sup>2</sup>
Laboratory Parameters				
Leukocytes (/mm <sup>3</sup> )	9580 (1630-27530)	9580 (5090-27530)	9610 (1630-30260)	0.69 <sup>1</sup>
Lymphocytes (/mm <sup>3</sup> )	1447.2+131.4	1663+201.5	1212.3 (81.5-4034)	0.09 <sup>1</sup>
D-dimer (mg/dL)	2,3(0.2-20)	1.39 (0.2-12.4)	5.06+1.07	<0.01 <sup>1</sup>
CRP (mg/L)	54.1 (4.5-472.8)	32,2 (4.5-472.8)	76.3+8.1	0.04 <sup>1</sup>
CT Value	22.72+0.99	23.26+1.22	22.3+1.51	0.64 <sup>3</sup>
Radiological Parameters				
Pneumonia	(52 26 %)	11 (50%)	15 (53.6%)	0.8 <sup>2</sup>

*Mann-Whitney Test, 2Chi Square Exact Test, 3Independent T-Test*

Table 1 also presents data on clinical, laboratory and radiological parameters based on the mild-moderate and critical-severity groups. The clinical parameters included are oxygen saturation, respiratory rate, and death outcome. Laboratory parameters shown included leukocyte count, absolute leukocyte count, d-dimer level, and CRP level. While the radiological parameters reported were the presence or absence of pneumonia which was assessed based on radiological assessment.

In table 1, it is known that oxygen saturation has a median value of 96% with a range of 30-99% and the respiratory rate has a median value of 26x/minute with a range of 18-36x/minute. Subjects with mild-moderate symptoms had higher oxygen saturation and lower respiratory rate which were statistically significant ( $p < 0.05$ )

compared to the critical-severe group. It is known that there were 8 (16%) research subjects who died during the hospitalization period with a significantly higher proportion in the critical weight group than the mild to moderate group ( $p < 0.05$ ).

In laboratory parameters, it is known that the leukocyte value is 9580/mm<sup>3</sup> with a range of 1630-27530/mm<sup>3</sup> and the average absolute lymphocyte value is 1447.2+131.4/mm<sup>3</sup>. In this study, there was no difference in the absolute leukocyte and lymphocyte values between subjects in the mild-moderate group and those in the severe-critical group. Inflammatory marker values obtained in this study showed D-dimer levels of 2.3 mg/dL with values ranging from 0.2 to 20 mg/dL and CRP 54.1 mg/L with values ranging from 4.5 to 472.8 mg/dL. L.

Comparison of D-dimer and CRP levels between the two groups showed statistically significant differences. D-dimer and CRP levels were found to be significantly higher in the critical-severe group than in the mild-moderate group ( $p < 0.05$ ). The CT-Value value of the SARS-CoV-2 PCR examination showed a CT Value of  $22.72 + 0.99$ , with the mean not different between the two groups ( $p > 0.05$ ).  
**Comparison of SARS-CoV-2 IgG Levels Between Groups of Disease Degrees**

Comparison of SARS-CoV-2 IgG Levels Patients with mild-moderate and severe-critical COVID-19 in this study were analyzed using the Mann-Whitney test because the data was not normally distributed. From the results of this analysis, it was found that SARS-CoV-2 IgG was not significantly lower in the mild-moderate group than in the critical-severe group. A comparison of SARS-CoV-2 IgG levels based on the degree of COVID-19 disease is presented in Table 2 below.

Table 2. Differences in IgG Levels in COVID-19 Patients based on Symptoms

SARS-CoV-2 IgG levels	Research Subjects (n=50)	Mild-moderate (n=22)	Critical-Heavy (n=28)	P
IgG Day 10 (AU/mL)	818.4 (7.4-54466.5)	249.1 (8.80-13475.8)	2039.3 (7.4-54466.5)	0.051 <sup>1</sup>

<sup>1</sup>Mann Whitney test

This study also sought the optimal cutoff point for SARS-CoV-2 IgG levels as a predictor in determining the severity of COVID-19 patients. From the ROC curve analysis, the cut point for SARS-

CoV-2 IgG levels was obtained in this study. Figure 1 below shows the cut point curve for IgG levels of SARS-CoV-2 in predicting the severity of COVID-19.

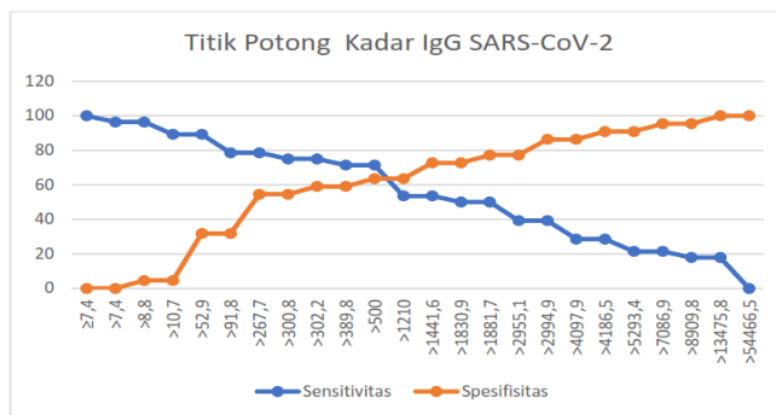


Figure 1. Graph of the intersection point of SARS-CoV-2 IgG levels with the severity of COVID-19

From the data analysis using the ROC curve in Figure 12, the cut point value for IgG SARS-CoV-2 levels in predicting the severity of COVID-19 disease is obtained, which is 500 AU/mL. At the cut point of 500 AU/mL, a sensitivity of 72.1% and a specificity of 64.5% were obtained. SARS-CoV-2 IgG levels >500 AU/mL increase the prevalence of COVID-19 disease severity. The following is an analysis of the relationship between SARS-CoV-2 IgG levels and the severity of disease in COVID-19 which can be seen in Table 4. In laboratory tests the results of high SARS-CoV-2 IgG levels (SARS-CoV-2 IgG levels  $\geq 500$  AU /mL) totaled 27 people, there were 19 patients

(67.9%) in the severe-critical disease group and 8 patients (36.4%) with mild-moderate disease. In samples with low levels of SARS-CoV-2 IgG (SARS-CoV-2 IgG < 500 AU/mL) there were 14 patients (64.6%) with mild-moderate disease, while 9 patients (32.1%) with critically severe degrees. A table was calculated to measure the relationship between high SARS-CoV-2 IgG levels and the degree of severity of COVID-19 (mild-moderate or severe-critical). It was found that high SARS-CoV-2 IgG levels were more than 500 AU/mL causing an increased risk of 3.69 (95% CI 1.13-11.98) times for severe-critical COVID-19.

Table 3. Relationship between SARS-COV-2 IgG Levels and COVID-19 Severity

SARS-CoV-2 IgG levels	Degree of Disease			Amount	p*	OR
	Critical-Heavy	Mild-Medium				
High SARS-COV-2 IgG ( $\geq 500$ AU/mL)	19 (67.9%)	8 (36.4%)	27 (54%)			3.69
Low SARS-COV-2 IgG (<500 AU/mL)	9 (32.1%)	14 (63.6%)	23 (46%)		0.027	1.13-11.98
Amount	28	22	50			

\*Chi-Square test

#### Multivariate Analysis

To find out the independent variables that can be used as predictors of the severity of COVID-19, a multivariate logistic regression analysis was carried out using the Backward LR method. The variables included in this multivariate analysis are variables that in the bivariate analysis have a p value <0.25. The independent variables

included in the multivariate analysis were age over 40 years, female gender, D-dimer level, absolute lymphocyte count <1500/mm<sup>3</sup>, D-dimer level >50 mg/dl, and CRP level >2 mg/L, and IgG level <500Au/mL. The results of the multivariate analysis for predictors of the severity of COVID-19 can be seen in Table 4 below.



**Table 4. Factors Predictors of COVID-19 Severity**

Predictors (n=50)	Coefficient	SE	Wald	df	p.s	Odds Ratio
Male gender	-2,277	0.798	8,13	9	1 0.004	0,103 (0,021-0,491)
IgG < 500 AU/mL	-1,499	0.757	3,92	2	1 0.048	0,517 (0,223-0,985)
D-Dimer	0.312	0.148	4,46	9	1 0.035	1,367 (1.023-1,826)
Constant	4,843	1,776	7,43	5	1 0.006	126,853

## DISCUSSION

### General Characteristics of Research Subjects

Based on the results of the study, more confirmed cases of COVID-19 being treated were found in the male sex, compared to the female sex. This data has the same trend as data published by WHO that the prevalence of confirmed cases of COVID-19 globally is reported with a percentage of 51% for men and 49% for women. Based on records from the Ministry of Health of the Republic of Indonesia (Kemenkes RI), confirmed cases of COVID-19 were found in 48.2% of women and 51.8% of men.

In the study, there were significant differences between the sexes in groups with mild-moderate and severe-critical disease. This research is in line with the research by Omran et al which reported that men are more susceptible to having a more severe degree of COVID-19 disease than women.

This finding relates to the direct link between androgen receptor activity involved in the transcription of the TMPRSS2 gene. In addition, male susceptibility is thought to be due to an X-linked genetic polymorphism between the androgen receptor and the ACE 2 gene locus. Estrogen and testosterone have different

immunoregulatory functions that may influence immune protection or disease severity. Estrogen that binds to estrogen receptors (ESR1/2) will inhibit the inflammatory process and prevent hyperinflammatory conditions which are associated with morbidity and mortality in hospitalized COVID-19 cases.

Patient comorbidities greatly affect the outcome of COVID-19. In this study, the most frequent comorbidities were Type 2 DM, namely 17 subjects (34%), followed by hypertension, namely 14 subjects (28%) and chronic failure disease in 4 subjects (8%). This is almost the same as the map of the distribution of COVID-19 disease in Indonesia where the highest comorbidity rates are hypertension 50.7%, DM 35.8% and chronic kidney disease 17.6%. COVID-19 mortality is around 10-14%, especially for patients over 40 years of age with co-morbidities such as heart disease, hypertension, kidney disease and diabetes.

The results showed that the absolute lymphocyte count was lower in the critical-severity group. This is also in line with several previously published studies by Yang L. et al (2020) which found that the number of lymphocytes correlated significantly with the severity of COVID-19. Jafarzadeh et al concluded that lymphopenia is a

predictor of severe COVID-19 and an absolute lymphocyte count of 1,200 AU/mL can predict the severity of the clinical outcome of COVID-19. Subgroup analysis showed that patients who died, ARDS, or patients who were treated in the intensive care unit had a low lymphocyte count, so it can be concluded that lymphopenia is associated with a severe degree of COVID-19.

#### Comparison Rate IgG SARS-CoV-2 Between Group Degree of Disease

The results of the study showed higher levels of IgG SARS-CoV-2 in the critical-severe group than the mild-moderate group. This difference was statistically significant in the multivariate analysis but not significantly different in the bivariate analysis. These results are slightly different from the hypothesis that the severity of SARS-CoV2 will lead to a stronger immune response resulting in higher SARS-CoV-2 IgG titers. Previous published studies have shown contradictory results.

Feng et al's study showed that there was no significant difference in SARS-CoV-2 IgG levels between patients with mild symptoms and patients who required mechanical ventilation. Another study by Marklund et al showed different results. Patients with critical severity tend to have significantly higher IgG levels than the mild-moderate group. In addition, critical-severe patients tend to have sero-conversion faster than the mild-moderate patient group (11 days vs 22 days).

In this study it was found that patients with mild-moderate symptoms had lower IgG levels of SARS-CoV-2 than patients with

critically severe symptoms. Similar findings have been documented for other coronavirus diseases (eg SARS and MERS) whose etiology involves a significant contribution of immunopathogenesis.

Mechanism underlying the relationship between anti-SARS-CoV-2 IgG levels and the severity of COVID-19 continues to be studied. Critical-severe COVID-19, caused by excessive inflammation and/or uncontrolled replication of SARS-CoV-2, can cause excessive antibody production.

Relationship between COVID-19 severity and SARS-CoV-2 viral load in plasma, nasopharyngeal, and sputum specimens was identified in a 2020 study by Fajnzylber et al. 58 Patients with severe-critical symptoms had higher viral loads than patients with mild-moderate symptoms, demonstrated that early symptoms of viral antigen counts may contribute to a stronger serological response. The results of this study indicate that the titer of specific IgG for SARS-CoV-2 depends on the severity of COVID-19.

Strong humoral response to SARS-CoV-2 may be related to the exaggerated immune response from severe-critical COVID-19, which includes a cytokine storm involving interleukin-1 (IL-1), IL-6, and interferon- $\gamma$ . Selective B-cell plasmablast amplification in patients with critical-severe COVID-19 may be associated with stronger SARS-CoV-2-specific humoral responses and reduced numbers of naïve B cells and memory B cells. Another potential mechanism could be the induction of a SARS-CoV-2 specific IgG response by prolonged enhanced B-cell receptor stimulation.

### Limitations

In this study, the IgG levels of SARS-CoV-2 were not significantly different based on statistical tests in bivariate analysis. However, in multivariate analysis it is known that SARS-CoV-2 IgG levels can affect the degree of disease. The limitations of this study are that the study was conducted in a cross-sectional manner and the subjects collected were not homogeneous so that further research should be carried out with a prospective cohort design with homogeneous initial patient data. Causative analysis can be done with a prospective cohort design.

### CONCLUSION

Serum IgG levels of SARS-CoV-2 were found to be significantly higher in patients with severe-critical symptoms than patients with mild-moderate symptoms in COVID-19 inpatients at Dr. Mohammad Hoesin Palembang.

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