THE COMPARISON OF CD8+ T CELL LEVELS AND SARS-CoV-2 IgG ANTIBODY POSSIBLE POSTING THE COVID-19 BOOSTER VACCINATION IN SURVIVAL AND NON-SURVIVAL OF COVID-19

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Abstract

Corona Virus Disease 19 (COVID-19) caused by SARS-CoV-2 (*Severe Acite Respiratory Syndrome Coronavirus* 2) is an outbreak/pandemic that appeared at the end of 2019. Efforts to deal with COVID-19 continue to be pursued, one of which is by vaccination which is believed to be an effective effort to break the chain of the spread of the virus, raise immunity in the community (*herd immunity*) and reduce morbidity/death rates. Vaccines will stimulate the specific immune system in the body. The cellular immune system will destroy the antigen and the humoral immune system will form a memory or immunity. The purpose of this study was to compare the levels of cellular (CD8+ T cells) and humoral (SARS-CoV-2 IgG antibodies) immune responses after administration of the COVID-19 *booster vaccine* in the survival group and the non-survival group of COVID-19. This study used an analytic observational design with a *cross sectional research design* . A sample of 78 people was divided into two groups, namely 39 survivals and 39 non-survivals. Sampling was carried out from March to July 2022. Samples were taken 6 months after the Moderna *booster vaccination* (third dose). Examination of CD8+ T cells used the *flow cytometry method* and examination of IgG antibodies used the CLIA (S-RBD) method. Data analysis used the *Independent T Test test* for CD8+ T Cell levels and the *Man Whitney test* for SARS-CoV-2 IgG antibody levels. From the results of the study, the average CD8+ T cell level in COVID-19 survivals was 520.13 ± 189.81 Cells/µL and in non-survivals was 631.87 ± 250.01 Cell/µL. The results of the IgG levels of SARS-CoV-2 in survivals have a median value of 1000 U/mL, a minimum value of 395 U/mL and a maximum value of >1000 U/mL while non-survivals have a median value of 1000 U/mL, a minimum value of 302 U/mL and a maximum value of >1000 U/mL. The conclusion of the study was that there was a significant difference in the levels of CD8+ T cells between the survivals and non-survivals groups with a significance value of 0.032 ($p<0.05$). The levels of IgG SARS-CoV-2 antibodies in the two study groups had the same median value due to the maximum detection capacity of the measuring method used.
Keywords: CD8+ T Cells, IgG-SARS-CoV-2 Antibody, COVID-19 Booster Vaccination.

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Corona Virus Disease 19 (COVID-19) caused by SARS-CoV-2 (*Severe Acite Respiratory Syndrome Coronavirus* 2) is an epidemic/pandemic that emerged at the end of 2019. Efforts to overcome COVID-19 continue to be pursued, one of which is by vaccination which is believed to be an effective effort to break the chain of virus spread, generate immunity in society (herd immunity) and reduce morbidity/death rates. Vaccines will stimulate the specific immune system in the body. The cellular immune system will destroy the antigen and the humoral immune system will form a memory or immunity. The purpose of this study was to compare the levels of cellular (CD8+ T cells) and humoral (SARS-CoV-2) immune responses after the administration of the COVID-19 booster vaccine in the survivals and non-survivals of COVID-19. This study used an analytic observational design with a cross-sectional research design. A sample of 78 people was divided into two groups, namely 39 survivals and 39 non-survivals. Sampling was carried out from March to July 2022. Samples were taken after 6 months of Moderna booster vaccination (third dose). Examination of CD8+ T cells using flow cytometry method and examination of IgG antibodies using the CLIA (S-RBD) method. Data analysis used Independent T Test for CD8+ T cell levels and Man Whitney test for SARS-CoV-2 IgG antibody levels. The results showed that the average CD8+ T cell level in COVID-19 survival was 520.13 ± 189.81 Cells/uL and in non-survivals was 631.87 ± 250.01 Cells/uL. The results of the SARS-CoV-2 IgG antibody levels in survivals had a median value of 1000 U/mL, a minimum value of 395 U/mL and a maximum value of >1000 U/mL, while non-survivals had a median value of 1000 U/mL, a minimum value of 302 U/mL and a maximum value of >1000 U/mL. The conclusion of the study was that there was a significant difference in the levels of CD8+ T cells in survivals and non-survivals with a significant value of 0.032 ($p<0.05$). The levels of IgG SARS-CoV-2 antibodies in the two study groups have the same median value due to the maximum detection capacity of the measuring method used
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1. Introduction

Corona Virus Disease 19 (COVID-19) is an outbreak/pandemic that appeared at the end of 2019, was first reported in the city of Wuhan, the capital of Hubei Province, China. (Elidiya, A. *et.al* , 2021). COVID-19 is caused by SARS-CoV-2 (*Severe Acite Respiratory Syndrome Coronavirus* 2) and WHO has declared COVID-19 a Global Pandemic. (Velavan & Meyer, 2020). The spread of SARS-CoV-2 is very fast. This can be seen from the number of cases which continue to increase and expand to cover many aspects of human life. Based on data from WHO, as of early January 2021, it was recorded that 235 countries had experienced the pandemic with 103 million sufferers spread across various countries. The death rate is also increasing very fast. As of 15 June 2021, there have been more than 175 million cases of COVID-19 including more than 3.8 million deaths reported in 221 countries. (Elidiya, A, *et.al* , 2021, Wu, Q, *et.al*, 2021). Efforts to tackle COVID-19 continue to be pursued with various strategies, one of which is vaccination. Vaccination is believed to be an effective effort to break the chain of transmission of the virus, raise immunity in the community (herd immunity) and reduce morbidity/death rates. Specifically for health workers, the Indonesian government has allocated a booster vaccine (third dose) which is expected to increase the body's immunity against the COVID-19 virus. (RI Ministry of Health, 2021). Vaccines stimulate the immune system in the body to fight antigens that enter the body. Vaccines contain viruses or their components that have been controlled or attenuated so that they are not causing pain and harmless. Vaccines will stimulate the specific immune system in the body. The cellular immune system will destroy the antigen and the humoral immune system will form a memory or immunity if the same antigen enters the body.

CD8+ T cells are cytotoxic T lymphocyte cells that play a role in cellular immunity as effectors to destroy cells infected with pathogens. SARS-CoV-19 IgG antibody is an immunoglobulin G (IgG) protein formed by plasma cells, due to the proliferative action of B cells when it binds to or comes in contact with a specific antigen of SARS-CoV-2. IgG is the main component of all immunoglobulins in the body and plays a role in long-term immunity. (Hadi, MI, *et.al*, 2020)

This study aims to compare the levels of cellular (CD8+ T cells) and humoral (SARS-CoV-2 IgG antibodies) immune responses after administration of the COVID-19 booster vaccine using the Moderna vaccine in survivals and non-survivals of COVID-19. Research regarding the effect of the third booster dose of vaccination on survivals and non-survivals of COVID-19 after 6 months of vaccination is still rare or has not been widely published.

2. Method

This research is an analytic observational study with a cross sectional research design. The research was conducted at Charitas Hospital Palembang, the study population was employees of Charitas Hospital Palembang. The research samples came from survivals and non-survivals of COVID-19 who had received the Moderna booster vaccine 6 months ago. The data collected is primary data which is directly taken through observation. Examination of CD8+ T cell levels was carried out in the Prodia laboratory using the *flow cytometry method* while examination of the levels of IgG SARS-CoV-2 antibodies was carried out in the Charitas Hospital Palembang laboratory using the CLIA method (S-RBD). Data analysis used the *Independent* T Test and the *Mann Whitney Test* .

3. Results

3.1 General Characteristics of Research Subjects

General characteristics of the subjects in this study included gender, age, body mass index (BMI), co-morbidities and the presence or absence of KIPI events. The research subjects were hospital employees who were divided into two groups, namely the group of survivals of COVID-19 and the group of non-survivals of COVID-19, each group consisting of 39 people. The results of the frequency distribution can be seen in the table below:

Table 1. Characteristics of Research Subjects

	Characteristics	Research subject				Total		p value
		survivals		Non Survivals				
		n	%	n	%	n	%	
1.	Gender							
	-Man	9	45	11	55	20	100	0.795
	-Woman	30	51.7	28	48.3	58	100	
2.	Age (Years)							
	-20-44 years	31	53.4	27	46.6	58	100	0.437
	-45-54 years	8	40	12	60	20	100	
3.	Body mass index							
	-Thin	1	100	0	0.00	1	100	
	-Normal	18	54.5	15	45.5	33	100	0.441
	-Fat	20	45.5	24	54.5	44	100	
4.	Concomitant							
	Diseases	5	63.5	3	37.5	8	100	0.711
	-There is	34	48.6	36	51.4	70	100	
	-Not							
5.	KIPI Post 6 Months							
	-There is	0	0.0	0	0.0	0	0.00	0.00
	-Not	39	100	39	100	78	100	

**Chi-Square Test*

From the sex data it was found that there were 20 male sex, consisting of 9 survivals (45%) and 11 non-survivals (55%) and 58 women, consisting of 30 survivals (51.7%) and 28 people are not survivals (48.3%). Gender data produces a p value of 0.795 (p> 0.05).

From the age data it is known that the research subjects consisted of 22-44 years of age as many as 58 people consisting of 31 survivals (53.4%) and 27 non-survivals (46.6%) and aged 45-54 years as many as 20 people consisting of 8 survivals (40%) and 12 non-survivals (60%). The average age of the research subjects for the survivals group was 36 years and for the non-survivals group was 38 years. Age data produces a p value of 0.437 (p> 0.05).

From the BMI data, research subjects were categorized into thin, normal, and obese levels. Overall there was 1 survival who was in the thin category, 33 people in the normal category consisting of 18 survivals (54.5%) and 15 non-survivals (45.5%) and 44 people who were in the fat category consisting of 20 survivals (45.5%)) and 24 non-survivals (54.5%). BMI data produces a p value of 0.441 (p>0.05).

From the data on comorbidities, it was found that 8 people had comorbidities consisting of 5 survivals (63.5%), 3 people with hypertension and 2 people with asthma and 3 people who were not survivals (37.5%) namely 1 person with asthma, 1 person with hypertension and 1 person with DM, and 70 people without comorbidities consisting of 34 survivals (48.6%) and 36 non-survivals (51.4%). Comorbid disease data yielded a p value of 0.711 (p>0.05).

From the data whether or not there were post-immunization adverse events (KIPI) experienced by research subjects 6 months after the booster vaccination It was obtained from filling out the questionnaire that all research subjects, both survivals and non-survivals, no longer experienced post-immunization adverse events.

3.2 Statistic analysis

Statistical analysis in this study was carried out using descriptive statistical procedures (mean, median, frequency, standard deviation, minimum and maximum). The normality test is carried out by the *Kolmogorov-Smirnov test* and followed by the *Independent T Test* if the data is normally distributed, and the *Mann Whitney Test* if the data is not normally distributed. 3.13.7 The characteristic data is followed by stratification analysis according to each group on CD8+ T cell levels and IgG antibody levels.

3.3 Overview of CD8+ T Cell Levels in Survivals and Non Survivals of COVID-19

In this study to measure levels CD8 + T cells in survivals and non-survivals the *flow cytometry* method was used by taking EDTA blood samples. The results of measuring CD8+ T cells in units of Cells/µL, the results can be seen in the table below:

Table 2. Comparison of Average CD8+ T Cell Levels

Variable	CD8+ T cells		
	Mean (Cells/µL)	SD	p-value
COVID-19 survivals	520.13	189.81	0.032
Non COVID-19 Survivals	631.87	250.01	

** T Test Independent*

Table 2 shows that the average CD8+ T cell level in survivals is 520.13 ± 189.81 Cells/µL, the mean CD8+ T cell level in non-survivals was 631.87 ± 250.01 Cell/µL. These results indicate that the average level of CD8+ T cells in the non-survival group was higher than the average level of CD8+ T cells in the survival group. To see a comparison of CD8+ T-cell levels in survivals and non-survivals, the *Independent T Test* was carried out, the test yielded a significance value of 0.032 (p<0.05) which indicated that there was a significant

difference in CD8+ T-cell levels in the survivals and non-survivals groups.

3.4 Overview of IgG Antibody Levels in Survivals and Non Survivals of COVID-19

In this study, to measure the levels of IgG SARS-CoV-2 antibodies in survivals and non-survivals, the CLIA method (S-RBD) was used by taking blood samples (serum). The results of measuring SARS-CoV-2 IgG antibodies in units of U/mL, the results can be seen in the table below:

Table 3. Comparison of Average SARS-CoV-2 IgG Antibody Levels

Variable	SARS CoV-2 IgG antibodies	
	Med (Min-Max) (U/mL)	p-value
COVID-19 survival	1000 (395-1000)	0.483
Non COVID-19 Survival	1000 (302-1000)	

**Mann Whitney test*

From table 3. it can be seen that the survivals sample has a median value of 1000 U/mL, with a minimum value of 395 U/mL and a maximum value of >1000 U/mL. The non-survival sample has a median value of 1000 U/mL with a minimum value of 302 U/mL and a maximum value of >1000 U/mL. To see the comparison of IgG antibody levels in survivals and non-survivals, the *Mann Whitney* statistical test was carried out, the test yielded a significance value of 0.483 ($p>0.05$) indicating that there was no significant difference in IgG antibody levels in the survivals and non-survivals groups.

3.5 Analysis of Sub-Group Characteristics of Research Subjects

Based on the characteristic data of the research subjects, it was followed by stratification analysis according to each group on CD8+ T cell levels and IgG antibody levels. Analysis used the T *Test Independent test* on CD8+ T Cell levels and used the *Mann Whitney test* on IgG antibody levels. The results of subgroup analysis can be seen in table 4 and table 5.

Table 4 Analysis of Sub-Group Levels of CD8+ Study Subjects

No	Characteristics/Groups	CD8+ T cells (Cells/uL)		p -*value*
		Survival	Non Survivals	
1	Gender			
	Man	623.44 ± 223.82	612 ± 313.81	0.704
	Woman	489.13 ± 170.54	639.68 ± 226.44	0.04
2	Age			
	20-44 Years	557.32 ±178.99	675.00 ± 268.07	0.083
	45-54 Years	376.00 ±168.51	534.83 ±176.73	0.036
3	BMI			
	Thin	-	-	-
	Normal	521.83 ± 162.55	614.33 ±197.75	0.178
	Fat	521.05 ± 219.72	642.83 ± 281.32	0.107
4	Concomitant Diseases			
	There is	693.40 ± 288.73	550.33 ± 19.42	0.602
	There isn't any	494.65 ±161.74	638.67 ± 259.28	0.012
5	KIPI			
	There is	-	-	-
	There isn't any	-	-	-

* *Independent Sample T Test*

Table 5. Subgroup Analysis of SARS-CoV-2 IgG Levels in Research Subjects

No	Characteristics/Groups	SARS-CoV-2 IgG Antibody (U/mL)		p -*value*
		Survival	Non Survivals	
1	Gender			
	Man	1000 (950-1000)	1000 (519-1000)	0.766
	Woman	1000 (345-1000)	1000 (302-1000)	0.633
2	Age			
	20-44 Years	1000 (637-1000)	1000 (302-1000)	0.287
	45-54 Years	1000 (395-1000)	1000 (357-1000)	0.851
3	BMI			
	Thin	-	-	-
	Normal	1000 (685-1000)	1000 (302-1000)	0.873
	Fat	1000 (395-1000)	1000 (357-1000)	0.318
4	Concomitant Diseases			
	There is	-	-	1,000
	There isn't any	1000 (395-1000)	1000 (302-1000)	0.545
5	KIPI			
	There isn't any	-	-	-

* *Mann Whitney test*

From table 4 above it can be seen that the male sex shows that the average CD8+ T-cell level in the male survivals group is higher than the male non-survivals group. The levels of CD8+ T cells in the male sex resulted in a significance value of 0.704 ($p>0.05$) which indicated that there was no significant difference in the levels of CD8+ T cells in the survivals and non-survivals groups in the characteristics of the male sex.

In the female sex, it was found that the average level of CD8+ T cells in the group of female non-survivals was higher than the group of female survivals. CD8+ T cell levels in the female sex resulted in a significance value of 0.04 ($p<0.05$). This shows that there are significant differences in the survivals' group and the non-survivals' group in terms of female sex characteristics.

The characteristic age of 20-44 years showed that the levels of CD8+ T cells in the non-survival group were higher than the survival group. The level of CD8+ T cells at the age of 20-44 years resulted in a significance of 0.083 ($p>0.05$), this indicated that there was no significant difference in the levels of CD8+ T cells in the survival and non-survival groups at the age of 20-44 years.

At the age of 45-54 years, the average CD8+ T-cell level was found to be higher in the non-survival group than in the survival group. The levels of CD8+ T cells at the age of 45-54 years resulted in a significance of 0.036 ($p<0.05$) indicating that there was a significant difference in the levels of CD8+ T cells in the survival and non-survival groups at the age of 45-54 years.

Based on BMI characteristics, it was found that the levels of CD8+ T cells in the lean group did not produce significance in statistical tests because the number of samples was only 1 (one) sample. In normal BMI, the levels of CD8+ T cells in the non-survival group were higher than in the survival group. The levels of CD8+ T cells in normal BMI yielded a significance value of 0.178 ($p>0.05$), this indicated that there was no significant difference in the levels of CD8+ T cells in the survivals and non-survivals of the normal BMI category.

In the obese category , the levels of CD8+ T cells in the non-survival group were found to be higher than the survival group. CD8+ T cell levels in obese IMT yielded a significance value of 0.107 ($p>0.05$), this indicated that there was no significant difference in CD8+ T cell levels in the survivals and non-survivals of the obese BMI category.

In the presence/absence of co-morbidities, the levels of CD8 T cells in the survivals group were higher than in the non-survivals group. CD8+ T-cell levels in samples with co-morbidities resulted in a significance value of 0.602 ($p>0.05$), this indicated that there was no difference in CD8+ T-cell levels in survivals and non-survivals with co-morbidities.

In the samples without co-morbidities, the average CD8+ T cells in the non-survival group were higher than the survival group. CD8+ T-cell levels in samples without co-morbidities resulted in a significance value of 0.012 ($p<0.05$) which indicated that there was a significant difference in CD8+ T-cell levels in the survivals group and non-survivals in the group without co-morbidities.

In the group with or without KIPI in the study sample, it was found that the CD8+ T-cell levels in the sample did not produce a significant value because all samples did not experience KIPI events after 6 months of booster vaccination.

From table 5 above it can be seen that in terms of sex characteristics, the average minimum level in the survivals group is higher than the minimum average level in

the non-survivals group. IgG antibody levels in the male sex characteristics yielded a significance value of 0.766 (p>0.05), this indicated that there was no significant difference in IgG antibody levels in the survival and non-survival groups in the male sex.

In the female sex, the average minimum level in the survivals group was higher than the non-survivals group's average minimum level. IgG antibody levels in the female sex characteristics yielded a significance value of 0.633 (p>0.05), this indicated that there was no significant difference in IgG antibody levels in the survival and non-survival groups in the female sex.

In terms of age characteristics, the average minimum level in the survivals group was higher than the non-survivals group's average minimum level. IgG antibody levels in the 20-44 year old characteristics yielded a significance value of 0.287 (p>0.05), this indicated that there was no significant difference in IgG antibody levels in the survival and non-survival groups in the 20-44 year age group.

In terms of age characteristics, the average minimum level in the survivals group was higher than the non-survivals group's average minimum level. IgG antibody levels in the 45-54 year old characteristics yielded a significance value of 0.851 (p>0.05), this indicated that there was no significant difference in IgG antibody levels in the survival and non-survival groups in the 45-54 year age group.

Based on BMI characteristics, the average minimum level in the survivals group was higher than the non-survivals group's average minimum level. IgG antibody levels in normal BMI yielded a significance value of 0.873 (p>0.05), this indicated that there was no significant difference in IgG antibody levels in the survivals and non-survivals groups in the group with normal BMI.

Based on BMI characteristics, the average minimum level in the survivals group was higher than the non-survivals group's average minimum level. IgG antibody levels in obese BMI yielded a significance value of 0.318 (p>0.05), this indicated that there was no significant difference in IgG antibody levels in the survivals and non-survivals groups in the group with obese BMI.

In the presence/absence of co-morbidities, it was found that at IgG antibody levels, samples with co-morbidities yielded a significance value of 1.00 (p>0.05) and samples without co-morbidities yielded a significance value of 0.545 (p>0.05). This shows that there is no difference in IgG antibody levels in samples with co-morbidities or without co-morbidities.

In the group with or without KIPI in the study sample, it was found that the IgG antibody levels in the samples did not produce a significant value because all samples did not experience KIPI.

4. Discussion

4.1 Characteristics of Research Subjects

Based on data on the characteristics of the research subjects, the gender distribution showed that there were more female research subjects compared to male research subjects. From the age data, the sample is dominated by samples aged 22-44 years rather than samples aged 45-54 years. Based on BMI, the sample data was dominated by samples with BMI in the fat category. Based on the presence or absence of co-morbidities, samples were dominated by samples without co-morbidities, while based on the presence or absence of KIPI, none of the study subjects experienced KIPI after 6 (six) months after the booster vaccine.

4.2 CD 8+ T Cell Levels in Survivals and Non Survivals of COVID-19

From the results of the study, it was found that the average CD8+ T cell level in the survivals group was 520.13±189.81 Cells/µL while the average CD8+ T cell level in the non-survival group was 631.87±250.01 Cell/µL. This shows that the average level of CD8+ T cells in the non-survival group was higher than the average level of CD8+ T cells in the survival group. Comparison of CD8+ T-cell levels in the survivals and non-survivals groups yielded a significance value of 0.032 ($p<0.05$) indicating that there was a significant difference in CD8+ T-cell levels in the survivals and non-survivals groups.

The results of the effectiveness of giving vaccines to individuals who have never been exposed to or infected with COVID-19 provide very good effectiveness results, as in a study conducted by Clarke, CL, *et.al*, 2021, which states that the results of the effectiveness of the immune response are 95%. This means that the immune response in those who are healthy provides protection against infection. In addition, research by Chen, Z., & John Wherry, E, 2020 states that vaccines provide 70% protection to individuals who are in good health or are not infected after administration of the vaccine.

Other results were obtained in the study of Reynolds, CJ, *et.al,* 2021 which analyzed the response of T cells and B cells after vaccination in those who had and had never been infected with SARS-CoV-2 before. The results of the study stated that after one dose of vaccination, individuals with previous infections showed increased T-cell immunity, memory B cell responses that secreted antibodies against the spike protein, and neutralizing antibodies that were effective against variants B.1.1.7 and B.1.351 whereas those who received one dose of vaccine without prior infection shows reduced immunity to the variant. Spike mutations B.1.1.7 and B.1.351 result in increased, abrogated, or unchanged T-cell responses, depending on *human leukocyte antigen* (HLA) polymorphisms.

In addition, specific T cells appear functionally in healthy patients so that the effectiveness of the vaccine is estimated at 80%, this is because adaptive immune cells are activated by a collection of S peptides which increase sequentially on days 7-10 after the first dose of vaccination. m-RNA vaccine induced SARS-CoV-2 specific T cells early and functionally efficiently both CD4+ and CD8+ which was temporarily appropriate with an initial vaccine efficacy of 80%, particularly a specific T cell response detectable on day 10 which was detectable in individuals with recovered from a previous SARS-CoV-2 infection. The Pfizer vaccination provides a fairly good booster rate of specific T cell response of 50%. (Kalimuddin, S, et.al, 2021)

Administering the vaccine to a healthy body results in high effectiveness at 70-95%, while COVID-19 survivals who receive a booster vaccine show a percentage of 50-93%. Patients who have been infected with COVID-19 tend to have weak CD4 and CD8 T cells after administration of the vaccine, this is because CD8 cytotoxic cells do not work specifically with *helper T cells* . (Alfatihah, B, 2021).

According to Sureshchandra S, *et.al*, 2021 the characteristics of cellular immunity induced by mRNA vaccines are still unclear. Early studies testing the efficacy of m-RNA vaccines have shown a strong antigen-specific Th1 response after 2 doses of the vaccine. Single-cell analysis suggested an expansion of activated CD4+ T cells (CD38+HLA-DR+) skewing towards Th1 and Th17 phenotypes, whereas the frequency of activated CD8+ T cells remained unchanged after vaccination.

m-RNA vaccines have shown significant protection against severe COVID-19 disease. Both mRNA vaccines induced strong, long-lasting neutralizing antibodies from 10 days and persisted for up to 8 months after the first dose of vaccination. The data showed that the titers after the first dose were comparable to those observed in recovered individuals, the titer level being significantly higher in the vaccinated group after the booster (Sureshchandra S, *et.al* , 2021).

4.3 SARS-CoV-19 IgG Antibody Levels in COVID-19 Survivals and Non Survivals

The results showed that the average IgG antibody level in the survivals group was 953.23 U/mL, with a median value of 1000 U/mL, a maximum value of >1000 U/mL and a minimum value of 395 U/mL, while the average IgG antibody level in the group non survivals was 911.69 U/mL with a median value of 1000 U/mL, a maximum value of >1000 U/mL and a minimum value of 302 U/mL. This shows that the average antibody level in survivals is slightly higher than the average IgG antibody level in non-survivals, although this is not statistically significant. This result is the same as the results of a study by Hutapea, R. D, 2021 which revealed that the average S-RBD SARS-CoV-2 antibody level was higher in subjects with a confirmed history of COVID-19 than in subjects without a confirmed history of COVID-19. Another study stated that there were reactive/seropositive results with a median IgG antibody level of 1951.5 AU/ml. The humoral response of IgG SARS CoV-2 antibodies to the majority of respondents showed positive results with varying ranges of values after 2 doses of vaccination (Irsan, A, *et.al* , 2022).

From the results obtained where IgG antibody levels can still be detected up to 6 months after vaccination, the vaccine plays an important role in the Moderna vaccine or m-RNA vaccine containing/based on RNA encoding the viral *spike protein* (S) from SARS-CoV-2 is very helpful in activating the immune response against the COVID-19 pathogen, where this vaccine has the ability to perform self-amplifying which makes extreme RNA replication occur in the cytosol of the body's cells, and will induce activation of B cells and T cells. (Elidiya, *et al,* 2021).

In the results of the analysis using the *Mann Whitney test* for IgG levels of SARS-CoV-2 antibodies, a significance value of IgG levels of SARS-CoV-2 was found to be p *value* = 0.483 (p>0.05). These results indicate that the levels of IgG SARS-CoV-2 antibodies did not have a significant difference in the COVID-19 survivals group and the non-survivals group. This is possibly caused by the Moderna vaccine which works effectively in groups of non-survivals of COVID-19 resulting in levels of humoral and cellular immunity that are almost the same as those of COVID-19 survivals/those who have had COVID-19. Moderna vaccine or mRNA-1273 which is an m-RNA type vaccine. This vaccine encodes the viral *spike* (S) protein of SARS-CoV-2. (Elidiya, *et.al* , 2021). The mRNA-1273 vaccine has a good safety profile and is very useful/efficacious in preventing COVID-19, after 6 months of follow-up, although there has been a gradual decline in vaccine efficacy. According to research, this vaccine is good at dealing with the spread of the Delta variant virus. This vaccine is currently approved or cleared for emergency use worldwide. (Thomas, SJ, *et.al,* 2021, Tartof, SY, *et.al,* 2020, Pilishvili, T, *et.al,* 2021).

4.4 Analysis of Research Characteristic Subgroups on Levels of CD8+ T Cells and SARS COV-2 IgG Antibodies

Gender Group

On the sex characteristics, there are differences in the immune response. The difference lies in the sex steroids especially testosterone, estradiol, and progesterone which affect the function of immune cells. Sex steroids alter the function of immune cells by binding to specific receptors that are expressed on various cells of lymphoid tissue as well as in circulating lymphocytes, macrophages, and DCs. Females show enhanced humoral and cellular immune responses to antigenic stimulation, vaccination, and infection than males. Both basal immunoglobulin (Ig) levels as well as antibody responses to viruses and vaccines are consistently higher in women than in men among young and old individuals. (Fink & Klein, 2015)

In the study, the average CD8+ T-cell level in the survivals group was higher than the non-survivals group, although there was no significant difference. This result is inversely proportional to the results in the female sex group where the average level of CD8+ T cells in the non-survival group was higher than the survival group and had statistically significant differences. This is in accordance with the research of Scully, EP., *et.al*, 2020 which revealed that biological sex affects innate and adaptive immune responses to self-antigens and foreign antigens, autoimmunity and in response to infections and vaccines. In addition, this study showed that the immune response in the form of the number of CD8+ T cells in female non-survivals had a more active response than in female survivals and the male group, both survivals and non-survivals. Studies have shown that women have greater numbers of CD3+ and CD4+ T cells and a higher CD4+/CD8+ ratio than men, whereas the frequency of CD8+ T cells and NK cells is greater in men. CD4+ and CD8+ T-cell activity after stimulation is often greater in women than in men. (Jacobsen H & Klein, SL, 2021).

According to de Vries, ST, *et.al*, 2022, the antibody response in women increases against viral infections and vaccinations. However, in this study, different results were obtained, namely antibody levels in males produced an average antibody value that was greater than that of females in both the survival and non-survival groups. However, the thing that needs attention is that the number of samples in this study was dominated by female samples in both survivals and non-survivals groups.

Age Group

The age group in this study was divided into 2 groups, namely the 20-44 year old group and the 45-54 year old group. CD8+ T cell levels in the 20-44 year old group had an average number of CD8+ T cells higher than the 45-54 year old group. This is in accordance with research by Otani, J, *et.al* , 2021 that age can also be a factor in obtaining immunity after COVID-19 vaccination. This study shows that age correlates with the amount of anti-SARS-CoV-2 IgG with higher IgG levels in younger participants. This is related to immune viability in younger populations compared to older populations, which has been demonstrated in previous studies of other vaccines. Younger individuals tend to have more T and B cells associated with adaptive immunity and active receptor reactions which have a greater association with higher IgG levels among younger individuals than in older individuals.

In the 45-54 year age group, the levels of CD8+ T cells in the non-survival group were found to be higher than the survival group and had a significant

significance value. This is in accordance with the study of Anggraini, DP, *et.al*, 2022 which revealed that there were statistically significant differences in the levels of CD8+ T cells in post-vaccination serum COVID-19 in the elderly and adults. Meanwhile, IgG antibody levels did not produce significant significance values. This is in line with the study of Rotty, I, *et.al*, 2022 that the formation of specific IgG antibodies in the elderly group requires a longer time than the non-elderly group.

IMT Group

In this study, in the BMI category, the sample group was dominated by fat BMI. The results of the average levels of CD8+ T cells and IgG antibodies in this study produced a significance value that was not significant. This study is in line with research by Anggraini, DP, *et.al*, 2022 which stated that there was no significant difference in IgG antibody levels after vaccination in BMI status. The immune response is a highly energy-dependent process, in which activated T cells increase glucose uptake and aerobic glycolysis for survival and function. Leptin is important for activated T cells to regulate glucose uptake and metabolism (Saucillo, DC, et.al, 2014).

Presence/absence of comorbid disease group

In this study, the majority of the samples were those who did not have comorbidities. The results of the mean CD8+ T-cell levels showed that there were significant differences in the group without co-morbidities. The levels of CD8+ T cells in the non-survival group were higher than the survival group and there was no significant difference in samples with comorbidities, this is in line with the study by Anggraini, DP, *et.al*, 2022 that comorbidities had no effect on post-vaccination CD8+ T cell results.

4.5 Research Weaknesses

In determining research subjects it is difficult to get samples that are completely free of COVID-19, because not all subjects exposed to COVID-19 show specific symptoms and carry out tests, so that in-depth examinations cannot be carried out or there is no medical record data that can be traced .

In examining the levels of IgG antibodies to SARS-CoV-2, it was only carried out up to the highest level >1000 U/mL, during the examination process no dilution was carried out on the samples due to limited funds. This has an impact on research results that are unable to more accurately describe the results of examinations on SARS-CoV-2 IgG antibody levels. Besides having an impact on the results, it also has an impact on the results of statistical analysis.

5. Conclusion

The conclusion of the study was that there was a significant difference in the levels of CD8+ T cells between the survivals and non-survivals groups with a significance value of 0.032 ($p<0.05$). The levels of IgG SARS-CoV-2 antibodies in the two study groups had the same median value due to the maximum detection capacity of the measuring method used.

BIBLIOGRAPHY

1. Alfatiah, B. (2021). Literature Review: Adaptive Immune Response to the Pfizer Vaccine in Patients with Covid-19. Publication manuscript. Aisyiyah University.

2. Anggraini, DP, Eddy Mart Salim, Nur Riviati, Erial Bahar, Syamsu Indra, Nova Kurniati, Ahmad Rasyid & Yuniza. (2022). The Role of Nutritional Status on SARS-CoV-2 IgG Levels After COVID-19 Vaccination in Palembang. Bioscientia Medicina : Journal of Biomedicine and Translational Research, 6(9), 2122–2126. https://doi.org/10.37275/bsm.v6i9.569

3. Anggraini, DP, Paramita, D., Ghanie, A., Ali, Z., Umi Partan, R., Kurniati, N., Indrajaya, T., Riviati, N., & Mart Salim, E. (2022). Clinical Effectiveness and Comparison of Humoral and Cellular Systems After COVID-19 Vaccination in Adults and Elderly in Palembang. thesis. Faculty of Medicine, Sriwijaya University.

4. Chen, Z., & John Wherry, E. (2020). T cell responses in patients with COVID-19. Nature Reviews Immunology, 20(9), 529–536. https://doi.org/10.1038/s41577-020-0402-6 .

5. Clarke, CL, Prendecki, M., Dhutia, A., Gan, J., Edwards, C., Prout, V., Lightstone, L., Parker, E., Marchesin, F., Griffith, M., Charif , R., Pickard, G., Cox, A., McClure, M., Tedder, R., Randell, P., Greathead, L., Guckian, M., McAdoo, SP ,Willicombe , M. (2021) . Longevity of SARS-CoV-2 immune responses in hemodialysis patients and protection against reinfection. Kidney International, 99(6), 1470–1477. https://doi.org/10.1016/j.kint.2021.03.009

6. de Vries, ST, Starokozhko, V., Schellens, IMM, Wijnans, L., Enzmann, H., Cavaleri, M., & Mol, PGM (2022). Attention for sex in COVID-19 trials: A review of regulatory dossiers. In BMJ Global Health, 7 (3). https://doi.org/10.1136/bmjgh-2021-008173 .

7. Elidiya, A., Abdul Nasser, G., Dhanu, I., Himayani, R., & Ismunandar, H. (2021). COVID-19 Vaccine: Literature Review. Medulla, 11(1), 141-144.

8. Fink, AL, & Klein, SL (2015). Sex and gender impact immune responses to vaccines among the elderly. In Physiology, 30(6), 408–416. https://doi.org/10.1152/physiol.00035.2015 .

9. Hadi, MI, Alamudi, MY, & Widiyanti, M. (2020). Basic Theory and Concepts of Immunology (E. Kusumawati, Ed.; 1st ed. , 1). Intimedia: Malang

10. Hutapea, Ratna. D. (2021). Analysis of spike-receptor binding domain antibody levels in inactivated virus severe acute respiratory syndrome coronavirus-2 vaccine recipients. Thesis. PPDS Clinical Pathology Study Program. Hasanuddin University Faculty of Medicine: Makassar.

11. Irsan, A., Mardhia, M., & Rialita, A. (2022). RESEARCH ARTICLES Consistency of SARS-CoV-2 Humoral Immune Response (IgG) Post-SARS-CoV-2 Vaccination in Health Workers. 45(2), 118–125. http://jurnalmka.fk.unand.ac.id .

12. Jacobsen, H., & Klein, S. L. (2021). Sex Differences in Immunity to Viral Infections. In Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.720952 .

13. Kalimuddin, S., Tham, CYL, Qui, M., de Alwis, R., Sim, JXY, Lim, JME, Tan, HC, Syenina, A., Zhang, SL, le Bert, N., Tan, AT , Leong, YS, Yee, JX, Ong, EZ, Ooi, EE, Bertoletti, A., & Low, JG (2021). Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. Med, 2(6), 682-688.e4. https://doi.org/10.1016/j.medj.2021.04.003

14. Republic of Indonesia Ministry of Health. (2021). The third dose of vaccination for all health workers, assistant health workers and support workers who work in health care facilities.

15. Otani, J., Ohta, R., & Sano, C. (2021). Association between immunoglobulin g levels and adverse effects following vaccination with the bnt162b2 vaccine among Japanese healthcare workers. Vaccines, 9(10). https://doi.org/10.3390/vaccines9101149

16. Pilishvili, T., Gierke, R., Fleming-Dutra, KE, Farrar, JL, Mohr, NM, Talan, DA, Krishnadasan, A., Harland, KK, Smithline, HA, Hou, PC, Lee, LC, Lim , SC, Moran, GJ, Krebs, E., Steele, MT, Beiser, DG, Faine, B., Haran, JP, Nandi, U., Schrag, SJ (2021). Effectiveness of mRNA COVID-19 Vaccine among US Health Care Personnel. New England Journal of Medicine, 385(25), e90. https://doi.org/10.1056/nejmoa2106599

17. Reynolds, CJ, Corinna Pade, Joseph M. Gibbons, David K. Butler, Ashley D. Otter, Katia Menacho, Marianna Fontana ,AngeliqueSmit, Jane E. Sackville-West, Teresa Cutino-Moguel, Mala K. Maini, Benjamin Chain, Mahdad Noursadeghi. (2021). Prior SARS-CoV-2 infection rescues B and T cell responses to variants after first vaccine dose. Science Journal, 372 (6549), 8–10.

18. Rotty, I., Kristanto, EG, Sekeon, S., Ekawardani, N., & Liwe, HR (2022). Formation of SARS-CoV-2 Specific Antibodies after vaccination. E-CliniC, 10 (1), 16. https://doi.org/10.35790/ecl.v10i1.37193

19. Saucillo, DC, Gerriets, VA, Sheng, J., Rathmell, JC, & MacIver, NJ (2014). Leptin Metabolically Licenses T Cells for Activation To Link Nutrition and Immunity. The Journal of Immunology ,192 (1), 136–144. https://doi.org/10.4049/jimmunol.1301158 .

20. Scully, EP, Haverfield, J., Ursin, RL, Tannenbaum, C., & Klein, S.L. (2020). Considering how biological sex impacts immune responses and COVID-19 outcomes. Nature Reviews Immunology, 20(7), 442–447. https://doi.org/10.1038/s41577-020-0348-8 .

21. Sureshchandra, S., Lewis, SA, Doratt, BM, Jankeel, A., Ibraim, IC, & Messaoudi, I. (2021). Single-cell profiling of T and B cell repertoires following the SARS-CoV-2 mRNA vaccine. JCI Insights, 6(24). https://doi.org/10.1172/jci.insight.153201 .

22. Tartof, SY, Slezak, JM, Fischer, H., Hong, V., Ackerson, BK, Ranasinghe, ON, Frankland, TB, Ogun, OA, Zamparo, JM, Gray, S., Valluri, SR, Pan, K ., Angulo, FJ, Jodar, L., & McLaughlin, JM (2021). Effectiveness of mRNA BNT162b2 COVID-19

vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. The Lancet, 398 (10309), 1407–1416. https://doi.org/10.1016/S0140-6736(21)02183-8

23. Thomas, SJ, Moreira, ED, Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, JL, Pérez Marc, G., Polack, FP, Zerbini, C., Bailey, R ., Swanson, KA, Xu, X., Roychoudhury, S., Koury, K., Bouguermouh, S., Kalina, W. v., Cooper, D., Frenck, RW, Jansen, KU (2021). Safety and Efficacy of the BNT162b2 mRNA COVID-19 Vaccine through 6 Months. New England Journal of Medicine, 385(19), 1761–1773. https://doi.org/10.1056/nejmoa2110345\

24. Velavan, TP, & Meyer, CG (2020). The COVID-19 epidemic. Tropical Medicine and International Health, 25(3), 278–280. https://doi.org/10.1111/tmi.13383

25. Wu, Q., Dudley, MZ, Chen, X., Bai, X., Dong, K., Zhuang, T., Salmon, D., & Yu, H. (2021). Evaluation of the safety profile of COVID-19 vaccines: a rapid review. BMC Medicine, 19(1). https://doi.org/10.1186/s12916-021-02059-5