

## Comparison of ABO Blood Group Antibody Titers in Elderly and Young Adult Patients

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### Abstract

Immunosenescence in the elderly decreases their antibodies, which may lead to weaker degree of agglutination formation that potentially causes ABO discrepancies. This may lead to misinterpretation of blood group in this group. This study aimed to determine whether there are differences in antibody titer levels and degree of agglutination formed when examining ABO blood groups in the elderly as compared to young adults. This was a cross-sectional study employing analytical observational methods. Data were collected prospectively from the Blood Services Unit of Dr. Hasan Sadikin General Hospital Bandung, Indonesia, between May 2022 and July 2022. The subjects included were 42 elderly participants and 42 young adults, grouped accordingly. The ABO blood typing was performed using the slide method, while anti-A and anti-B titers were measured by serial two-fold dilution using the tube method. A significant difference in ABO blood group antibody titer levels was observed between elderly and young adult groups (median: 6 vs 64,  $p < 0.001$ ). Antibody titers were lower in the elderly across blood groups A (median: 8 vs 64,  $p < 0.001$ ), B (median: 8 vs 64,  $p < 0.001$ ), and O (median: 4 vs 64,  $p < 0.001$ ). The degree of agglutination was lower in the elderly (2+) compared to young adults (4+). On average, antibody titers in elderly individuals were approximately tenfold lower than those in young adults across all three blood groups. The reduced degree of agglutination further supports the diminished antibody response in the elderly group.

**Keywords:** ABO blood group, aged, antibodies, young adults

### Introduction

Aging causes many changes in the physiological systems of the body. One of the important changes resulting from aging occurs in the body's immune system.<sup>1</sup> In the elderly it is known that their immune system decreases, which causes an increase in the elderly's vulnerability to infectious diseases, degenerative diseases, autoimmune diseases and malignancies. A decrease in the immune system in the elderly is called immunosenescence.<sup>2</sup> This condition causes a decrease in the innate and adaptive immune response. One of the most influential responses of aging to the immune system is a decreased ability to produce antibodies. The

ability to differentiate, proliferate, and activate memory B cells decreases with increasing age, so the antibodies produced have a shorter duration of response than at a young age.<sup>3</sup>

In clinical practice, antibody activity is essential for ABO blood grouping, particularly in reverse typing, which detects plasma antibodies against A and B antigens. A reduction in antibody levels among the elderly may impair reverse typing, potentially leading to ABO discrepancies. The decrease in antibodies formed in the elderly can affect the reverse typing examination, causing a discrepancy between forward typing and reverse typing. This condition is known as ABO discrepancy, which can cause errors in the interpretation of blood groups which then lead to the administration of inappropriate blood groups which further leads to transfusion reactions.<sup>4</sup>

Transfusion is frequently required in elderly patients due to multiple comorbidities and higher surgical rates, with a reported

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transfusion prevalence of up to 62%. Some reasons for transfusion in elderly patients include the presence of comorbid conditions and the higher frequency of surgical procedures, making transfusions more common in older adults compared to younger individuals.<sup>5</sup> The high number of transfusion requests in the elderly shows the importance of appropriate procedures in carrying out transfusions to avoid transfusion reactions. ABO blood group examination using reverse typing is one of the precautions that can be taken to confirm blood group. Antibodies in the patient's plasma are reacted with a suspension of erythrocytes A, B, and O from healthy individuals whose blood group is known, then the agglutination formed is assessed. Weakened antibodies in the elderly can affect the degree of agglutination formed. Doubts in detecting agglutination can lead to misinterpretation of blood groups, resulting in discrepancies and can have life-threatening consequences for the patient.<sup>6</sup> Research shows that the incidence of ABO discrepancies due to decreased or weak antibodies reaches 9.2%, which often occurs among the elderly, newborns, and immunodeficiency patients.<sup>7</sup>

Weakening ABO antibody levels in the elderly can be identified by checking antibody titer levels and comparing them with healthy young adults. Several studies have mentioned how important it is to estimate ABO blood group antibody titer levels. ABO blood group titer levels can also provide information on the relative amount of antibodies present in the serum, and this information is very important in certain cases such as in operations requiring emergency blood transfusions, hemolytic disease in newborns, and in patients undergoing organ transplants.<sup>8</sup>

This study aims to determine whether there are differences in antibody titer levels and the degree of agglutination during ABO blood group testing between elderly individuals and healthy young adults at Dr. Hasan Sadikin General Hospital Bandung. The findings are expected to inform safer blood bank practices by enabling more accurate antibody detection and minimizing ABO discrepancies in elderly patients.

## Methods

This study used an analytical observational approach with a cross-sectional design. Data collection was conducted prospectively from May to July 2022 at the Blood Services Unit of Dr. Hasan

Sadikin General Hospital Bandung, Indonesia. A total of 84 participants were included, consisting of 42 elderly individuals (aged  $\geq 60$  years) and 42 young adults (aged 26–45 years), each group meeting the age criteria established by the Ministry of Health of the Republic of Indonesia. Ethical approval was granted by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital (registration number: LB.02.01/X.6.5/305/2022).

The inclusion criteria were elderly outpatients treated at the geriatric clinic and young adult patients undergoing medical check-ups who had normal hematology results, including at least ten parameters: hemoglobin, hematocrit, leukocytes, erythrocytes, platelets, MCV, MCH, MCHC, RDW-SD, and RDW-CV. The determination of the age limits of the two groups is based on the age criteria set by the Ministry of Health of the Republic of Indonesia. The exclusion criteria for this study were elderly outpatients aged  $\geq 60$  years with comorbid malignancies, autoimmune diseases, infections (seen from the patient's history of laboratory examination results in the last three months via the laboratory information system), and incomplete variable data.

ABO blood group typing was performed using forward and reverse typing via the slide method. The calculation of anti-A and anti-B titers was carried out using a serial two-fold dilution using 0.9% NaCl using the tube method. Then the examiner assesses the agglutination reaction in the tube that still provides the highest level of dilution and determines the patient's antibody titer level. Titer level checks were carried out on research samples with blood groups A, B, and O. Observations were carried out with the assistance of one observer other than the researcher to assess the degree of agglutination and antibody titer. A suitability test was carried out using the Cohen's Kappa test to assess the results of the examination between the researcher and the observer.

All data were recorded in Microsoft Excel. Subject characteristics were summarized using frequency distribution tables. Differences in antibody titer levels between the elderly and young adult groups were analyzed using the Mann-Whitney test. Statistical analysis was conducted using SPSS version 25.

## Results

Table 1 shows that the elderly group was predominantly male (76.2%), while the young

**Table 1 Characteristics of Research Subjects**

Variables	Elderly (n=42) n (%)	Young Adults (n=42) n (%)	p-value
Sex			<0.001 <sup>a</sup>
Male	32 (76.2)	12 (28.6)	
Female	10 (23.8)	30 (71.4)	
Age (year)			
Mean ± SD	67.5±5.1	34.1±4.8	<0.001 <sup>b</sup>
Blood Group			1.000 <sup>c</sup>
A	11 (26.2)	11 (26.2)	
B	13 (31.0)	15 (35.7)	
O	18 (42.9)	16 (38.1)	
Degree of Agglutination in Reverse Typing			<0.001 <sup>c</sup>
1+	2 (4.7)	0 (0.0)	
2+	28 (66.7)	0 (0.0)	
3+	12 (28.6)	8 (19.0)	
4+	0 (0.0)	34 (81.0)	

<sup>a</sup>Test for different gender proportions using the Chi-Square test; <sup>b</sup>Test for differences in age means using the Mann-Whitney test; <sup>c</sup>Test for different blood group proportions and degrees of agglutination using the Kolmogorov-Smirnov test

**Table 2 Comparison of ABO Blood Group Antibody Titers between Elderly and Young Adults**

ABO Antibody Titer Levels	Elderly n=42	Young Adults n=42	p-value
ABO Blood Group			
Median (Min-Max)	6 (2-8)	64 (32-128)	<0.001 <sup>a*</sup>
<32	42 (100)	0 (0)	<0.001 <sup>b*</sup>
≥32	0 (0)	42 (100)	
A Blood Group (n=11)			
Median (Min-Max)	8 (2-8)	64 (32 - 128)	<0.001 <sup>a*</sup>
<32	13 (100)	0 (0)	<0.001 <sup>b*</sup>
≥32	0 (0)	16 (100)	
B Blood Group (n=13) (n=16)			
Median (Min-Max)	8 (4-8)	64 (32-128)	<0.001 <sup>a*</sup>
<32	13 (100)	0 (0)	<0.001 <sup>b*</sup>
≥32	0 (0)	16 (100)	
O Blood Group (n=18, 15)			
Median (Min-Max)	4 (2-8)	64 (32-64)	<0.001 <sup>a*</sup>
<32	18 (100)	0 (0)	<0.001 <sup>b*</sup>
≥32	0 (0)	15 (100)	

<sup>a</sup>Mann Whitney, <sup>b</sup>Chi Square

adult group was predominantly female (71.4%). The average age was 67.5 years in the elderly group and 34.1 years in the young adult group. The proportion of blood groups A, B, and O in the two groups showed no significant difference. However, the results obtained from this study show that the blood group O dominated the blood group of the research subjects.

Based on the degree of agglutination, the elderly group is dominated by 2+ with a few showing a degree of 3+. However, in young adults, the degree of agglutination is dominated by agglutination degree 4+. The results of the average ABO blood group antibody titer in elderly patients and young adults showed significant results, that the average antibody titer in the elderly was 10 times lower than in young adults. Between reader 1 and reader 2, a conformity test was carried out between the antibody titer readings using the Kappa test, and a value of 0.817 was obtained, which means a strong agreement with a p-value <0.001.

The results of the analysis in Table 2 show that there are differences in ABO blood group antibody titer levels between the elderly and healthy young adults at Dr. Hasan Sadikin General Hospital Bandung (median: 6 vs 64,  $p < 0.001$ ). Antibody titer levels were lower in the elderly compared to young adults in both blood groups A (median: 8 vs 64,  $p < 0.001$ ), B (median: 8 vs 64,  $p < 0.001$ ), and O (median: 4 vs 64,  $p < 0.001$ ).

## Discussion

In this study, the elderly group was predominantly male, which contrasts with findings from Madyaningrum et al. who reported that 51.6% of elderly outpatient visits were female.s. In this research, several factors influence the number of visits to elderly hospital clinics in Indonesia, including the presence or absence of health insurance, where at that time the BPJS national health insurance had only been running for one year. Other factors include economic status, religion, level of education, level of awareness of personal health conditions, and comorbidities suffered by the patient.<sup>9</sup> Further research is needed to determine the number of patient visits and the factors that influence visits at the elderly clinic at Dr. Hasan Sadikin General Hospital Bandung.

In this study, differences were found in the degree of agglutination formed in the reverse typing examination. The degree of agglutination in the elderly is lower with the average

agglutination being 2+, which can be seen from the formation of several small clumps with clear areas around them. In contrast, young adults have an agglutination average of 4+, which appears as large lumps. Erythrocyte agglutination can take place in two stages. The first stage is that the antibodies bind to the surface of the erythrocytes. This is then continued with the second stage, in which the antibodies interact with erythrocytes so that adjacent cells agglutinate. The first stage of agglutination is influenced by temperature, medium pH, antibody affinity constant, incubation time, ionic strength in the medium, and antibody-antigen ratio. The second stage of agglutination is influenced by the distance between cells, the charge of the molecules in the suspension, membrane deformity, membrane surface molecules, and molecular structure.<sup>6</sup>

In this studyslide method, the degree of agglutination is influenced by the antibody-antigen ratio, especially since immunosenescence occurs in the elderly. The ratio of antigen and antibody is very important in determining the strength of the reaction. The more antibodies that bind to the antigen on the surface of the erythrocytes, the stronger the reaction will be. Weak or missing antibodies found in old age (elderly) can cause the agglutination that forms to become weak.<sup>6</sup> In accordance with the results of this study, the antibody titer in elderly patients is 10 times lower than in young adults, so the degree of agglutination formed is also lower.

This research is in accordance with the research by Saidin, et al. which examined ABO antibody levels from 311 donor samples. It was found that the levels of anti-A and anti-B titers were low in people aged over 50 years, with an average titer < 1:64. The highest titer levels were found in the age range 18-29 years with an average titer  $\geq 1:64$ .<sup>10</sup> The average age of young adults in this study was 34 years with an average titer of 1:60. Meanwhile, elderly people under 65 years of age have an average titer of 1:6. This occurs because, in the humoral immune system, the production of B lymphocytes gradually decreases due to aging so the ability to produce antibodies also decreases. The ability to differentiate, proliferate, activation of memory B cells decreases and when antibodies are produced, the duration of presence of the antibody response in the elderly is shorter than in the young.<sup>11</sup>

Beyond aging, anti-ABO levels may also be influenced by ethnicity, diet, lifestyle, and environmental exposure. The high titer of ABO antibodies in Asian and African populations has

been suggested to be caused by mosquito bites and parasitic intestinal infections. Many bacteria, viruses, and parasites have surface antigens that resemble ABO blood group antigens. Chronic exposure to these pathogens can lead to the production of cross-reactive antibodies, boosting ABO antibody levels. Asian and African countries have large forest areas, increasing the risk of mosquito bites. The study by Kannan et al., found a significant relationship between types of diet and high antibody titers. Donors with a vegetarian diet have a higher chance of obtaining high titer antibodies ( $\geq 1:64$ ) compared to those with a mixed diet ( $< 1:64$ ).<sup>12</sup> However, food consumption factors were not examined in this study.

This study has several limitations. Factors that could influence ABO antibody titers—such as vaccination history, infection status, pregnancy history in female subjects, and methodological limitations of the antibody titer assay—were not considered. Furthermore, the use of the conventional tube method, which is inherently subjective, may have introduced variability in interpretation across examiners.<sup>13</sup> In conclusion, the ABO antibody titer in the elderly is 10 times lower and the degree of agglutination formed is also lower compared to young adults. Although this does not cause differences in blood group interpretation, it is still important for elderly patients to have their ABO blood group checked using reverse typing to prevent misinterpretation due to low ABO antibody titers.

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