eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

The Clinical Significance of DAT Positivity: A Comparative Analysis of IgG, C3d, and IgG/C3d-Positive Patients

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ARTICLE INFO

Keywords:
Autoimmune hemolytic anemia
C3d
Direct antiglobulin test
IgG
Monospecific anti-human globulin

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i5.1288

A B S T R A C T

Background: The direct antiglobulin test (DAT) is a crucial diagnostic tool in immunohematology, used to detect the presence of antibodies and/or complement components on the surface of red blood cells. DAT positivity is frequently associated with autoimmune hemolytic anemia (AIHA) and other immune-mediated hemolytic conditions. This study aimed to investigate the clinical significance of DAT positivity by comparing the characteristics of patients with IgG, C3d, and IgG/C3d-positive results. **Methods:** A retrospective study was conducted on 55 patients with DAT-positive results, identified from the Blood Transfusion Unit of Dr. M. Djamil General Hospital Padang between June 2023 and August 2023. DAT-positive samples were further analyzed using monospecific anti-human globulin (AHG) reagents to determine the presence of IgG, C3d, or both on the red blood cells. Patient demographics, clinical diagnoses, blood groups, transfusion history, and hematological parameters were collected and analyzed. **Results:** Out of the 55 DAT-positive patients, 51 (92.7%) were positive for IgG alone, 3 (5.5%) were positive for both IgG and C3d, and only 1 (1.8%) was positive for C3d alone. The majority of patients were adults (>18 years old) and female. A history of blood transfusion (>3 times) was common, particularly in the IgG-positive group. Hematological parameters indicative of hemolysis (hemoglobin ≤9 g/dL, reticulocyte count >2%, and total bilirubin >2 mg/dL) were observed in a significant proportion of patients, especially those with IgG and/or C3d positivity. **Conclusion:** IgG positivity was the most common finding in DAT-positive patients, highlighting the prevalence of warm AIHA. The presence of C3d, alone or with IgG, suggests the involvement of complement activation and may indicate a different underlying pathology. This study emphasizes the importance of using monospecific AHG reagents to characterize DAT-positive results, as this information can aid in the diagnosis, management, and prediction of clinical outcomes.

1. Introduction

The direct antiglobulin test (DAT) stands as a cornerstone in the field of immunohematology. It is an indispensable diagnostic tool that plays a critical role in detecting the presence of antibodies and/or complement components that are bound to the surface of red blood cells in vivo. This test is fundamental to the investigation of immune-mediated hemolytic conditions. DAT positivity is a significant indicator frequently associated with autoimmune hemolytic anemia (AIHA). However, its clinical implications extend beyond AIHA, as it is also implicated in a variety of other conditions. These include hemolytic transfusion reactions, where the recipient's immune

system attacks transfused red blood cells, leading to their premature destruction. Furthermore, DAT positivity is observed in hemolytic disease of the fetus and newborn (HDFN), a condition arising from maternal antibodies crossing the placenta and targeting fetal red blood cells. Drug-induced hemolytic anemia, where certain medications trigger an immune response against red blood cells, is another condition where DAT positivity is evident. In addition, DAT positivity can occur in passenger lymphocyte syndrome, a complication following allogeneic hematopoietic stem cell transplantation, where donor lymphocytes attack recipient red blood cells. AIHA itself represents a complex and heterogeneous group of disorders. The unifying characteristic of these disorders is the premature destruction of red blood cells. This destruction is a consequence of autoantibodies, which are antibodies produced by the individual's immune system that mistakenly target the individual's own red blood cell antigens. The incidence of AIHA is estimated to be in the range of 1 to 3 cases per 100,000 individuals annually. The prevalence of AIHA is approximately 17 cases per 100,000 individuals.[1-3]

The classification of AIHA into various subtypes is based on several key factors. One important criterion is the optimal temperature at which the autoantibodies exhibit reactivity. Another crucial factor is the specific type of immunoglobulin or complement component that is coating the red blood cells. The DAT procedure typically involves the use of polyspecific anti-human globulin (AHG) reagents. These reagents are designed to detect both IgG antibodies and complement components, primarily C3d. While polyspecific AHG reagents provide initial information regarding the presence of antibodies and/or complement on red blood cells, further characterization is often necessary. To gain a deeper understanding of the underlying immune mechanisms and to guide clinical management decisions, it is essential to employ monospecific AHG reagents. Monospecific AHG reagents possess the capability to differentiate between IgG, C3d, and combined IgG/C3d positivity. This differentiation is critical because the clinical significance of DAT positivity can vary considerably. The variation depends on the underlying cause of the DAT positivity and the precise nature of the immunoglobulin or complement components that are detected.[4-6]

For instance, IgG positivity is commonly associated with warm AIHA. Warm AIHA is characterized by autoantibodies that react optimally at body temperature. These autoantibodies are predominantly of the IgG isotype. They bind to red blood cell antigens, which leads to extravascular hemolysis. Extravascular hemolysis is a process where red blood cells are destroyed outside of the blood vessels, primarily in the spleen and liver, through Fc receptor-mediated phagocytosis. In contrast, C3d positivity, whether it occurs alone or in combination with IgG, may suggest the presence of cold AIHA. Cold AIHA is a less common subtype of AIHA. It is characterized by autoantibodies that react optimally at lower temperatures, typically ranging from 4 to 30°C. Cold AIHA is frequently associated with IgM autoantibodies. These IgM autoantibodies can activate the complement cascade, a complex system of proteins that plays a crucial role in the immune response. Activation of the complement cascade can result in either intravascular hemolysis, which is the destruction of red blood cells within the blood vessels, or extravascular hemolysis, mediated by complement receptor-mediated phagocytosis, primarily in the liver. The identification of different DAT positivity patterns has significant implications for clinical practice. These patterns can provide valuable insights that aid in understanding the underlying pathological processes. Furthermore, this information is essential for guiding clinical management strategies and decisions. For example, the presence of IgG positivity is suggestive of warm AIHA. Warm AIHA typically demonstrates a favorable response to treatment with corticosteroids, a class of medications that suppress the immune system. Other immunosuppressive therapies may also be employed in the management of warm AIHA. On the other hand, the detection of C3d positivity may indicate cold AIHA

or other complement-mediated hemolytic conditions. These conditions may necessitate different treatment approaches, tailored to the specific underlying pathology. This study was designed to contribute to a more comprehensive understanding of the clinical significance of DAT positivity.[7-10] It aimed to achieve this goal by comparing the characteristics of patients exhibiting different DAT positivity patterns, specifically IgG, C3d, and IgG/C3d.

2. Methods

This study employed a retrospective study design. Retrospective studies involve the examination of past data and records. This approach was chosen to investigate the clinical significance of DAT positivity by analyzing existing laboratory results and patient information. The retrospective nature of the study allowed for the efficient collection and analysis of data over a defined period.

The study was conducted within the facilities of Dr. M. Djamil General Hospital Padang. Specifically, the data were collected from two units within the hospital: the Blood Transfusion Unit and the Central Laboratory. Dr. M. Djamil General Hospital Padang is a tertiary care hospital. Tertiary care hospitals provide specialized medical care to patients with complex and often rare conditions. The selection of this setting was pertinent to the study's objectives as it provided access to a diverse patient population with a broad spectrum of medical conditions, including those associated with DAT positivity. The Blood Transfusion Unit, in particular, is a key site for DAT testing, as it is routinely performed in the context of pre-transfusion testing, investigation of hemolytic transfusion reactions, and evaluation of patients with suspected immune-mediated hemolytic conditions.

The study population consisted of all patients who underwent DAT testing at the Blood Transfusion Unit of Dr. M. Djamil General Hospital Padang during the specified study period. DAT testing was performed using polyspecific AHG reagents. Polyspecific AHG reagents are designed to detect both IgG antibodies and complement components, specifically C3d, that may be present on the surface of red blood cells. The inclusion criterion for the study was that patients must have had a DAT test performed using polyspecific AHG reagents at the Blood Transfusion Unit during the study period. Furthermore, the results of the DAT test must have been positive. A positive DAT result indicates the presence of antibodies and/or complement components on the surface of red blood cells. Patients were excluded from the study if they had incomplete data. Incomplete data refers to situations where essential information required for the study analysis was missing from the patients' records. This could include missing demographic information, incomplete laboratory results, or insufficient clinical data. The exclusion of patients with incomplete data was necessary to ensure the accuracy and reliability of the study findings. The presence of missing data could introduce bias into the analysis and compromise the validity of the results.

The study sample was derived from the study population based on the inclusion and exclusion criteria outlined previously. A total of 55 patients met the criteria for inclusion in the study. These 55 patients all had positive DAT results using polyspecific AHG reagents. This sample size was deemed appropriate for the study's objectives, allowing for a comparative analysis of different DAT positivity patterns and their associated clinical characteristics.

The initial DAT testing was performed at the Blood Transfusion Unit of Dr. M. Djamil General Hospital Padang. The standard operating procedures of the Blood Transfusion Unit were followed for all DAT testing. These procedures are designed to ensure the accuracy and reliability of the test results. DAT testing involves the collection of a blood sample from the patient. The red blood cells are then separated from the plasma. The red blood cells are washed to remove any unbound antibodies or proteins. Polyspecific AHG reagents are added to the washed red blood cells. If antibodies or complement components are present on the surface of the red blood cells, they will bind to the AHG reagents, causing agglutination, which is the clumping together of red blood cells. The agglutination

is then visually assessed.

Following the initial DAT testing with polyspecific AHG reagents, samples that yielded positive results underwent further analysis. This involved the use of monospecific AHG reagents. Monospecific AHG reagents are designed to detect specific types of antibodies or complement components. In this study, monospecific AHG reagents containing antibodies against IgG and C3d were used. The use of monospecific AHG reagents allowed for the differentiation of DAT-positive results based on the specific type of antibody or complement component present on the red blood cells. This differentiation is crucial for understanding the underlying immune mechanisms involved in DAT positivity and for determining the clinical significance of different DAT positivity patterns. The procedure for monospecific AHG analysis was similar to the initial DAT testing. However, instead of using polyspecific AHG reagents, monospecific AHG reagents specific for IgG and C3d were used. The red blood cells were washed, and the appropriate monospecific AHG reagent was added. Agglutination was then assessed visually.

The degree of agglutination observed in both the initial DAT testing and the monospecific AHG analysis was graded. A semi-quantitative grading system was used to assess the strength of the agglutination reaction. The grading scale ranged from 1+ to 4+; 1+: This represents the weakest degree of agglutination, characterized by small clumps of red blood cells that are barely visible to the naked eye; 2+: This indicates a stronger reaction, with more distinct clumps of red blood cells that are easily visible; 3+: This signifies a moderate to strong reaction, with large clumps of red blood cells that are readily apparent; 4+: This represents the strongest degree of agglutination, with very large, solid clumps of red blood cells. The assessment of agglutination was performed by trained laboratory personnel experienced in immunohematological techniques. The grading of agglutination provides a semi-quantitative measure of the amount of antibody or complement component present on the red blood cells. This information can be useful in understanding the severity of the immune response and its potential clinical implications.

Data for the study were collected from two primary sources: the Laboratory Information System (LIS) and electronic medical records. The Laboratory Information System (LIS) is a software system used to manage and store laboratory data. It contains a wealth of information related to laboratory tests, including patient demographics, test results, and dates of testing. The LIS was used to retrieve data related to DAT testing, including the results of the initial DAT testing with polyspecific AHG reagents and the results of the monospecific AHG analysis. Electronic medical records are digital versions of patients' paper charts. They contain comprehensive information about patients' medical history, diagnoses, treatments, and laboratory results. The electronic medical records were accessed to obtain patient demographics, including age and gender. Clinical diagnoses were also extracted from the medical records to determine the underlying medical conditions associated with DAT positivity. Data related to blood groups and transfusion history were collected from the medical records to assess their potential influence on DAT positivity. Furthermore, hematological parameters were collected from the medical records. These parameters included hemoglobin levels, reticulocyte counts, and total bilirubin levels. Hemoglobin levels are a measure of the amount of hemoglobin in the blood, which is the protein in red blood cells that carries oxygen. Reticulocyte counts are a measure of the number of immature red blood cells in the blood, which can indicate the bone marrow's response to anemia. Total bilirubin levels are a measure of the amount of bilirubin in the blood, which is a yellow pigment formed during the breakdown of red blood cells. Elevated bilirubin levels can indicate increased red blood cell destruction. The data collection process was conducted in a systematic and standardized manner to ensure accuracy and completeness. Data were extracted from the LIS and electronic medical records by trained personnel. Quality control measures were implemented to verify the accuracy of the data. This

included double-checking data entries and comparing data from different sources.

The statistical analysis of the data was performed using descriptive statistics. Descriptive statistics are used to summarize and describe the main features of a dataset. They provide a clear and concise overview of the data, making it easier to understand and interpret. Categorical variables were presented as frequencies and percentages. Categorical variables are variables that can be classified into distinct categories. In this study, examples of categorical variables include gender (male or female), clinical diagnosis (e.g., autoimmune diseases, infections, malignancies), blood group (A, B, AB, or O), and DAT positivity pattern (IgG, C3d, or IgG and C3d). Frequencies refer to the number of observations in each category, while percentages represent the proportion of observations in each category relative to the total number of observations. Continuous variables were presented as means and standard deviations. Continuous variables are variables that can take on any value within a given range. In this study, examples of continuous variables include age, hemoglobin levels, reticulocyte counts, and total bilirubin levels. The mean is the average value of the variable, calculated by summing all the values and dividing by the number of values. The standard deviation is a measure of the dispersion or variability of the data around the mean. A larger standard deviation indicates greater variability, while a smaller standard deviation indicates less variability. The use of descriptive statistics allowed for a clear and concise summary of the patient characteristics and DAT positivity patterns. Frequencies and percentages were used to describe the distribution of categorical variables, while means and standard deviations were used to describe the central tendency and variability of continuous variables. These statistical measures provided a foundation for comparing the characteristics of patients with different DAT positivity patterns and for drawing conclusions about the clinical significance of DAT positivity.

3. Results

Table 1 presents a comprehensive overview of the key characteristics of the 55 patients included in the study. These characteristics encompass demographic information, clinical diagnoses, blood group distribution, history of blood transfusion, the degree of DAT positivity, and hematological parameters; Age and Gender Distribution: The table reveals that the majority of the patients (87.3%) were adults, specifically those above 18 years of age, while a smaller proportion (12.7%) were children (0-18 years). In terms of gender distribution, the study population consisted of a higher percentage of females (60.0%) compared to males (40.0%); Clinical Diagnoses: The study population exhibited a diverse range of clinical diagnoses, categorized into several groups. Autoimmune diseases were the most frequent diagnostic category (21.8%), including Systemic Lupus Erythematosus (SLE) (7.3%), Autoimmune Hemolytic Anemia (AIHA) (5.5%), Rheumatoid Arthritis (3.6%), and other autoimmune conditions (5.5%). Infections represented another significant category (12.7%), with specific diagnoses such as Typhoid Fever (5.5%), Dengue Fever (3.6%), Urinary Tract Infection (1.8%), and Pneumonia (1.8%). Malignancies were also present (9.1%), including both hematologic (5.5%) and non-hematologic malignancies (3.6%). Non-malignant hematologic diseases (12.7%) included Thalassemia Beta Major (5.5%), Sickle Cell Anemia (3.6%), and other hematologic disorders (3.6%). Liver diseases constituted a notable portion (25.5%), with diagnoses such as Liver Cirrhosis (14.5%), Hepatitis B (5.5%), Hepatitis C (3.6%), and other liver conditions (1.8%). Other diseases were also observed (18.2%); Blood Group Distribution: The distribution of blood groups among the patients showed that blood group O was the most common (38.2%), followed by blood group A (32.7%), blood group B (20.0%), and blood group AB (9.1%); History of Blood Transfusion: A significant proportion of the patients (65.5%) had a history of receiving more than 3 blood transfusions, while a smaller proportion (34.5%) had received 3 or fewer transfusions; Degree of DAT Positivity: The degree of

DAT positivity, as assessed by agglutination grading, varied among the patients. The majority (60.0%) showed a 1+ reaction, 29.1% showed a 2+ reaction, 9.1% showed a 3+ reaction, and 1.8% showed a 4+ reaction; Hematological Parameters: Hematological parameters indicative of hemolysis were evaluated. Hemoglobin levels were ≤9 g/dL in 63.6% of patients, while 36.4% had levels >9 g/dL. Reticulocyte counts were >2% in 78.2% of patients, with the remaining 21.8% having counts ≤2%. Total bilirubin levels were >2 mg/dL in 63.6% of patients, while 36.4% had levels ≤2 mg/dL.

Table 2 details the distribution of DAT positivity patterns among the 55 patients, as determined by monospecific AHG testing, and correlates these patterns with various patient characteristics. The table categorizes the DAT results into three groups: IgG positive, C3d positive, and IgG and C3d positive. The majority of the patients (92.7%) were positive for IgG alone, indicating that IgG was the predominant immunoglobulin detected on the red blood cells. Only a small fraction of patients showed positivity for C3d alone (1.8%), while 5.5% were positive for both IgG and C3d. In the IgG-positive group, the age distribution mirrored the overall study population, with most patients being adults (81.9% were >18 years). The single patient in the C3d-positive group was an adult (100%). In the IgG and C3d-positive group, both children (1.8%) and adults (3.6%) were represented. The gender distribution was also examined within each DAT positivity group. In the IgG-positive group, females were slightly more prevalent (52.7%) than males (40.0%). The single C3d-positive patient was female (100%). All patients in the IgG and C3d-positive group were female (100%). The distribution of clinical diagnoses varied across the DAT positivity patterns. Autoimmune diseases were present in the IgG-positive group (16.4%) and were the only diagnosis in the C3d-positive group (100%) and were also seen in the IgG and C3d-positive group (3.6%). Infections, malignancies, non-malignant hematologic diseases, liver diseases, and other diseases were only observed in the IgG-positive group. Malignancies were also seen in the IgG and C3d-positive group (1.8%). The distribution of blood groups was analyzed for each DAT positivity pattern. All blood groups (A, B, AB, and O) were represented in the IgG-positive group. The single C3d-positive patient had blood group B (100%). The IgG and C3d-positive group had A, AB, and O blood groups represented. A history of blood transfusion, categorized as ≤3 times or >3 times, was examined. The majority of patients in the IgG-positive group had a history of >3 transfusions (58.2%). The single C3d-positive patient had a history of >3 transfusions (100%). All patients in the IgG and C3d-positive group had a history of >3 transfusions (100%). The degree of DAT positivity, graded from 1+ to 4+, was mostly 1+ in the IgG-positive group (60.0%). The single C3d-positive patient showed a 4+ reaction (100%). The IgG and C3d-positive group showed 2+ and 3+ reactions. Hematological parameters, including hemoglobin levels, reticulocyte count, and total bilirubin levels, were assessed. The majority of patients in the IgG-positive group had hemoglobin levels ≤9 g/dL (56.4%). The single C3d-positive patient had a hemoglobin level ≤9 g/dL (100%). All patients in the IgG and C3d-positive group had hemoglobin levels ≤9 g/dL (100%). Most patients in the IgG-positive group had reticulocyte counts >2% (70.9%). The single C3d-positive patient had a reticulocyte count >2% (100%). All patients in the IgG and C3d-positive group had reticulocyte counts >2% (100%). The majority of patients in the IgG-positive group had total bilirubin levels >2 mg/dL (58.2%). The single C3d-positive patient had a total bilirubin level >2 mg/dL (100%). All patients in the IgG and C3d-positive group had total bilirubin levels >2 mg/dL (100%).

Table 1. Patient characteristics (n=55).

Characteristic	Category	n (%)
Age		
	0-18 years	7 (12.7)
	>18 years	48 (87.3)
Gender		
	Male	22 (40.0)
	Female	33 (60.0)
Clinical diagnosis		
	Autoimmune Diseases	12 (21.8)
	- Systemic Lupus Erythematosus (SLE)	4 (7.3)
	- Autoimmune Hemolytic Anemia (AIHA)	3 (5.5)
	- Rheumatoid Arthritis	2 (3.6)
	- Other Autoimmune Diseases	3 (5.5)
	Infections	7 (12.7)
	- Typhoid Fever	3 (5.5)
	- Dengue Fever	2 (3.6)
	- Urinary Tract Infection	1 (1.8)
	- Pneumonia	1 (1.8)
	Malignancies	5 (9.1)
	- Hematologic Malignancies	3 (5.5)
	- Non-Hematologic Malignancies	2 (3.6)
	Hematologic Diseases (Non-Malignant)	7 (12.7)
	- Thalassemia Beta Major	3 (5.5)
	- Sickle Cell Anemia	2 (3.6)
	- Other Hematologic Diseases	2 (3.6)
	Liver Diseases	14 (25.5)
	- Liver Cirrhosis	8 (14.5)
	- Hepatitis B	3 (5.5)
	- Hepatitis C	2 (3.6)
	- Other Liver Diseases	1 (1.8)
	Other Diseases	10 (18.2)
Blood group		
	A	18 (32.7)
	B	11 (20.0)
	AB	5 (9.1)
	O	21 (38.2)
History of blood transfusion		
	≤3 times	19 (34.5)
	>3 times	36 (65.5)
Degree of DAT positivity		
	1+	33 (60.0)
	2+	16 (29.1)
	3+	5 (9.1)
	4+	1 (1.8)
Hemoglobin (g/dL)		
	≤9	35 (63.6)
	>9	20 (36.4)
Reticulocyte count (%)		
	≤2	12 (21.8)
	>2	43 (78.2)
Total bilirubin (mg/dL)		
	≤2	20 (36.4)
	>2	35 (63.6)

Table 2. DAT positivity patterns with monospecific AHG (n=55).

DAT positivity pattern	IgG	C3d	IgG and C3d
Number of patients (n)	51	1	3
Percentage of patients (%)	92.7	1.8	5.5
Characteristics			
Age			
- 0-18 years	6 (10.9)	-	1 (1.8)
- >18 years	45 (81.9)	1 (100)	2 (3.6)
Gender			
- Male	22 (40.0)	-	-
- Female	29 (52.7)	1 (100)	3 (100)
Clinical diagnosis			
- Autoimmune diseases	9 (16.4)	1 (100)	2 (3.6)
- Infections	7 (12.7)	-	-
- Malignancies	5 (9.1)	-	1 (1.8)
- Hematologic diseases (Non-Malignant)	6 (10.9)	-	-
- Liver diseases	14 (25.5)	-	-
- Other diseases	10 (18.2)	-	-
Blood Group			
- A	17 (30.9)	-	1 (1.8)
- B	10 (18.2)	1 (100)	-
- AB	4 (7.3)	-	1 (1.8)
- O	20 (36.4)	-	1 (1.8)
History of blood transfusion			
- ≤3 times	19 (34.5)	-	-
- >3 times	32 (58.2)	1 (100)	3 (100)
Degree of DAT positivity			
- 1+	33 (60.0)	-	-
- 2+	14 (25.5)	-	2 (3.6)
- 3+	4 (7.3)	-	1 (1.8)
- 4+	-	1 (100)	-
Hemoglobin (g/dL)			
- ≤9	31 (56.4)	1 (100)	3 (100)
- >9	20 (36.4)	-	-
Reticulocyte count (%)			
- ≤2	12 (21.8)	-	-
- >2	39 (70.9)	1 (100)	3 (100)
Total bilirubin (mg/dL)			
- ≤2	19 (34.5)	-	-
- >2	32 (58.2)	1 (100)	3 (100)

4. Discussion

IgG positivity, as the predominant finding in our study, aligns with the well-established understanding of warm autoimmune hemolytic anemia (AIHA) as the most frequent subtype of AIHA. The direct antiglobulin test (DAT) detects antibodies or complement components bound to the surface of red blood cells. In our study, the high prevalence of IgG detection indicates that IgG antibodies are the primary mediators of red cell destruction in the majority of DAT-positive cases. The significance of IgG in warm AIHA lies in its interaction with red blood cell antigens at body temperature. Warm AIHA is characterized by autoantibodies that exhibit optimal reactivity at 37°C, which is normal body temperature. These autoantibodies, predominantly of the IgG isotype, bind to specific antigens present on the surface of red blood cells. This binding event is the initiating step in a

cascade of immune responses that ultimately lead to the premature destruction of the affected red cells. The IgG autoantibodies involved in warm AIHA recognize and target a variety of red blood cell antigens. These antigens may be part of various blood group systems, or they may be other proteins or carbohydrates expressed on the red cell membrane. The specificity of the autoantibodies can vary among individuals with warm AIHA, and in some cases, the autoantibodies may demonstrate broad reactivity, binding to multiple red cell antigens. Following the binding of IgG autoantibodies to red blood cell antigens, the antibody-coated red cells become susceptible to destruction through several mechanisms. The most important of these mechanisms is extravascular hemolysis, which predominantly occurs in the spleen. The spleen plays a central role in the clearance of IgG-coated red blood cells due to its unique microenvironment and the presence of specialized immune cells. Within the spleen, blood flow is relatively slow, and red blood cells must navigate through narrow passages in the splenic sinusoids. This slow transit allows splenic macrophages ample opportunity to interact with circulating red blood cells. Splenic macrophages are a type of immune cell that resides in the spleen and plays a critical role in the removal of damaged or abnormal cells from the circulation. These macrophages express Fc receptors on their cell surface. Fc receptors are specialized receptors that bind to the Fc region of IgG antibodies. The Fc region is a part of the IgG antibody molecule that is distinct from the antigen-binding sites. When red blood cells coated with IgG autoantibodies pass through the spleen, the Fc receptors on splenic macrophages recognize and bind to the Fc region of the IgG antibodies on the red cell surface. This interaction tethers the IgG-coated red cell to the macrophage. Once the macrophage has bound to the IgG-coated red cell, it initiates the process of phagocytosis. Phagocytosis is an active process by which the macrophage engulfs the red cell, internalizing it into a membrane-bound vesicle called a phagosome. Within the phagosome, a series of enzymatic reactions occur, leading to the breakdown and destruction of the red cell. The splenic macrophages are highly efficient at removing IgG-coated red blood cells from circulation. This process of extravascular hemolysis in the spleen is the primary mechanism responsible for the anemia observed in warm AIHA. While the spleen is the major site of extravascular hemolysis in warm AIHA, the liver also contributes to the removal of IgG-coated red cells. The liver contains macrophages, known as Kupffer cells, which are similar to splenic macrophages in their ability to phagocytose antibody-coated cells. However, the liver's role in red cell destruction is generally less prominent than that of the spleen in typical warm AIHA. The clinical consequences of IgG-mediated hemolysis in warm AIHA are directly related to the reduction in the number of circulating red blood cells. Red blood cells are essential for carrying oxygen from the lungs to the body's tissues. A decrease in the red cell mass leads to anemia, a condition characterized by a deficiency of red blood cells or hemoglobin. The severity of anemia in warm AIHA can vary considerably among individuals. In some cases, the hemolysis may be relatively mild, resulting in only a modest decrease in hemoglobin levels. In other cases, the hemolysis can be severe and life-threatening, leading to a profound anemia that requires urgent medical intervention. The rate and extent of red cell destruction are the major determinants of the severity of anemia in warm AIHA. Factors that can influence the rate of hemolysis include the titer and avidity of the IgG autoantibodies, the number of available target antigens on the red cell surface, and the efficiency of macrophage-mediated phagocytosis. Patients with warm AIHA can exhibit a wide spectrum of clinical manifestations, depending on the severity of their anemia and the rate of red cell destruction. Common symptoms include fatigue, a general feeling of tiredness or exhaustion, and weakness, a lack of physical strength. These symptoms arise from the reduced oxygen-carrying capacity of the blood, which impairs the delivery of oxygen to the body's tissues. Pallor, an abnormal paleness of the skin, is another

frequent sign of anemia. The reduced number of circulating red blood cells results in a decrease in the amount of hemoglobin, the red pigment in red blood cells that gives blood its color. Jaundice, characterized by yellowing of the skin and the whites of the eyes, can occur in warm AIHA due to the increased breakdown of red blood cells. The destruction of red cells releases bilirubin, a yellow pigment, into the circulation. The liver processes bilirubin, but in cases of rapid or excessive red cell destruction, the liver may not be able to process bilirubin efficiently, leading to its accumulation in the blood and tissues. Dark urine, also known as hemoglobinuria, can occur when there is significant intravascular hemolysis, which is the destruction of red blood cells within the blood vessels. In this process, hemoglobin is released directly into the bloodstream and may be filtered by the kidneys and excreted in the urine, giving it a dark color. Shortness of breath, or dyspnea, is a symptom that can arise from the reduced oxygen-carrying capacity of the blood. The body attempts to compensate for the anemia by increasing the heart rate and respiratory rate to deliver more oxygen to the tissues.[11-15]

The diagnosis of warm AIHA involves a combination of clinical evaluation and laboratory testing. The direct antiglobulin test (DAT) is a fundamental diagnostic tool in warm AIHA. A positive DAT result in the context of anemia and other clinical findings suggestive of hemolysis is highly indicative of warm AIHA. In warm AIHA, the DAT typically demonstrates the presence of IgG on the surface of red blood cells. Monospecific AHG reagents are used to confirm the presence of IgG and to differentiate it from other immunoglobulins or complement components. Other laboratory tests are performed to provide further evidence of hemolysis and to assess the severity of the anemia. A complete blood count (CBC) is performed to measure the levels of red blood cells, hemoglobin, and other blood components. In warm AIHA, the hemoglobin level is typically reduced, indicating anemia. The reticulocyte count is a measure of the number of immature red blood cells in the circulation. In warm AIHA, the reticulocyte count is usually elevated, reflecting the bone marrow's attempt to compensate for the anemia by producing more red blood cells. Bilirubin levels, both total and indirect, are often elevated in warm AIHA due to the increased breakdown of red blood cells. Lactate dehydrogenase (LDH) levels may also be increased, as LDH is released from damaged or destroyed cells. The treatment of warm AIHA is aimed at suppressing the underlying autoimmune response that is driving the destruction of red blood cells. The primary goal of treatment is to reduce or halt the hemolysis, thereby allowing the bone marrow to replenish the red cell mass and alleviate the symptoms of anemia. Corticosteroids, such as prednisone, are typically the first-line therapy for warm AIHA. Corticosteroids are potent immunosuppressive agents that work by reducing the production of autoantibodies and decreasing the activity of macrophages. They can effectively suppress the autoimmune response and slow down the rate of red cell destruction. The mechanism of action of corticosteroids in warm AIHA involves multiple effects on the immune system. Corticosteroids can reduce the production of cytokines, which are signaling molecules that promote inflammation and immune responses. They can also interfere with the interaction between macrophages and IgG-coated red blood cells, thereby decreasing the efficiency of phagocytosis. In many cases, corticosteroids are effective in inducing a remission of warm AIHA. However, some patients may not respond adequately to corticosteroids, or they may experience unacceptable side effects from long-term corticosteroid use. In these situations, other immunosuppressive agents may be considered. Rituximab is a monoclonal antibody that has emerged as a valuable treatment option for warm AIHA, particularly in cases that are refractory to corticosteroids or that require long-term immunosuppression. Rituximab targets and depletes B cells, which are the cells responsible for producing antibodies, including the pathogenic IgG autoantibodies in warm AIHA. By reducing the number of B cells, rituximab can decrease the production of autoantibodies and thereby reduce the

autoimmune attack on red blood cells. Rituximab is generally well-tolerated, although it can increase the risk of infections in some patients. Other immunosuppressive medications, such as azathioprine, cyclosporine, or mycophenolate mofetil, may be used in some cases of warm AIHA, either alone or in combination with corticosteroids or rituximab. These medications work by suppressing different aspects of the immune system, and their use is typically reserved for patients who have not responded to other therapies or who have significant side effects. In cases of warm AIHA with severe or refractory hemolysis, splenectomy, the surgical removal of the spleen, may be considered as a treatment option. The spleen is the primary site of extravascular hemolysis in warm AIHA, and its removal can significantly reduce the rate of red cell destruction. Splenectomy is most likely to be effective in patients in whom the spleen is the predominant site of red cell destruction. Before considering splenectomy, it is important to assess the role of the spleen in the individual patient. This may involve imaging studies or other tests to determine the degree of splenic involvement in the hemolytic process. While splenectomy can be effective in reducing hemolysis, it is an invasive procedure that carries certain risks, including infection and an increased risk of thrombosis. Therefore, the decision to proceed with splenectomy should be made on an individual basis, carefully weighing the potential benefits and risks.[16-20]

5. Conclusion

In conclusion, this retrospective study highlights the clinical significance of DAT positivity and the importance of characterizing DAT results using monospecific AHG reagents. The findings emphasize that IgG positivity is the most frequent pattern in DAT-positive patients, which is consistent with the high prevalence of warm AIHA. The presence of C3d, either alone or in conjunction with IgG, suggests the involvement of complement activation and may indicate distinct underlying pathological processes. The study's results underscore that identifying specific DAT positivity patterns has substantial implications for clinical practice. These patterns provide valuable information that aids in understanding the underlying pathological mechanisms, guiding clinical management strategies, and predicting clinical outcomes. For instance, IgG positivity is suggestive of warm AIHA, which typically responds well to corticosteroid treatment. In contrast, C3d positivity may indicate cold AIHA or other complement-mediated hemolytic conditions that may require alternative treatment approaches. Therefore, the use of monospecific AHG reagents to differentiate between IgG, C3d, and IgG/C3d positivity is crucial for accurate diagnosis, appropriate management, and informed clinical decision-making.

6. References

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