

DIRECT AND INDIRECT COOMBS TESTS: CLINICAL AND SEROLOGICAL CORRELATION AT A TERTIARY CARE ACADEMIC BLOOD CENTRE

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Abstract

Background: The Coombs test, or antiglobulin test, detects autoimmune diseases like hemolytic anemias. It uses antigen-antibody reactions with patients' RBCs and donors' sera (DCT) or patients' sera and donors' RBCs (ICT). It's vital for diagnosing AIHA, pregnancy-related hemolysis, drug-induced hemolysis, HDFN, and blood transfusion compatibility. **Materials and Methods:** In this study, we analyzed data from the Coombs tests performed over a five-year period from May 2019 to April 2023 at the Blood Centre, Sharda Hospital, School of Medical Sciences and Research, Sharda University. A total of 361 cases were examined, and clinical profiles were obtained from medical records. The aim was to correlate the test results with specific clinical diagnoses. **Results:** Our findings revealed a wide age range among the 361 patients, with 247 cases (65.3%) for DCT and 131 cases (34.6%) for ICT. Of the 15 DCT-positive cases, 12 were diagnosed with AIHA, while the remaining two had thyroiditis. The ICT-positive case was also associated with AIHA. These results highlight the importance of considering clinical and serological features when interpreting positive DCT results, as various factors, including infections, medications, and autoimmune conditions, can influence the test outcome. **Conclusion:** The Coombs test is a vital diagnostic tool in hemolytic anemias, particularly AIHA. However, interpreting the results requires a comprehensive evaluation of clinical data and patient history to ensure accurate diagnosis and appropriate management.

Keywords: Direct Antiglobulin Test, Indirect Antiglobulin Test, In Vivo Hemolysis, Autoimmune Hemolytic Anemia, Thyroid Lesions, Tuberculosis.

INTRODUCTION

Coombs test, known as antiglobulin test and is a diagnostic method used for identifying autoimmune diseases. [1] It is one of the commonest immune-haematological investigations performed in the blood bank. The basic principle of this test is antigen-antibody reaction, performed on patient's RBCs and donors' sera for direct test or patients' sera and donors RBCs for indirect Coomb's test. [2,3] The conditions like autoimmune haemolytic anaemias, pregnancy, drug induced haemolysis, haemolytic disease of foetus and new-born (HDFN) and compatibility testing prior to blood transfusion are some situations utilizing Coombs Testing for diagnosis.

AIHA is characterized by increased red cell destruction due to autoantibodies directed against self-antigens on red cells. [4] However, the presence of red cell coated immunoglobulins does not always indicate the presence of haemolysis. A combination of clinical and laboratory evidences of haemolysis is necessary to ascertain the

diagnosis of autoimmune haemolysis. The authors tabulated the data of the Coombs test performed at the Blood Centre, Sharda Hospital for the period of 5 years from May 2019 to April 2023 and correlated with clinical data available about the cases. The aim was to determine the specific clinical diagnosis with respect to the positive test results obtained.

MATERIALS AND METHODS

The Blood Centre, Sharda Hospital, School of Medical Sciences and Research, Sharda University collected data of a period of 5 years from May 2019 to April 2023 for the both direct and indirect Coombs test performed as part of routine investigative work up. A total of 361 cases were tabulated. Clinical profile of the patients was obtained from Medical Records and evaluated. All clinical information particularly with respect to Autoimmune Hemolytic anemias (AIHA) or having disorders with autoimmune spectrum like thyroiditis etc. was recorded. The data was tabulated and descriptive statistics applied on the results.

RESULTS

In this study encompassing 361 patients ranging from 1 month to 65 years of age, a total of 378 requisitions for Coombs testing were received at the blood center. (Table 1) Out of these, 247 (65.3%) were directed towards the Direct Coombs Test (DCT), while 131 (34.6%) were for the Indirect Coombs Test (ICT), with some cases requiring both. (Table 2). Notably, 12 cases exhibited DCT positivity in the context of Autoimmune Hemolytic Anemias (AIHA), while 2 cases revealed different diagnoses such as thyroiditis. The demographic profile of patients revealed variations across age groups and gender, emphasizing the need for a comprehensive assessment when interpreting Coombs test results, especially in the context of AIHA and related disorders. (Table 3)

Table 1: Demographic Profile of Patients' Sample Received for Coombs Testing

Age Class Interval (Years)	Female	Male	DCT	ICT
0-10	46	22	88	40
11-20	53	15	50	22
21-30	90	13	47	34
31-40	26	19	37	14
41-50	32	10	15	13
51-64	23	12	10	8
Total	270	91	247	131

Table 2: Positive Results with Respect to Age for the Coombs Testing

Age Range (Years)	DCT Positive	DCT Negative	ICT Positive	ICT Negative
0-10	02	86	-	40
11-20	04	46	01	21
21-30	05	42	-	34
31-40	02	35	-	14
41-50	01	14	-	13
51-64	01	09	-	08
Total	15	232	01	130

Table 3: Correlation of Clinical Diagnosis with Respect to Direct and Indirect Coombs Test Results

Clinical diagnosis	DCT Positive (No. of cases, n)	DCT Negative (No. of cases, n)	ICT Positive (No. of cases, n)
Autoimmune Hemolytic Anemias	12	5	1
Other Disorders Coexisting with Anemia (Thyroid Lesions etc.)	2	-	-
Tuberculosis	1		

DISCUSSION

DCT is one of the most commonly performed investigations to diagnose auto-immune aetiology as a part diagnostic work-up for anaemia. It is a simple, quick, and inexpensive test. However, false positivity of DCT may occur even without haemolysis. Studies have reported a high percentage of positive DCT in patients with a variety of acute illnesses. In some studies, an incidence of positive DCT was observed in 1 to 15% of hospitalized patients without obvious features of haemolysis.⁵⁻⁸ Drugs, immunoglobulins (IVIGs) and anti-thymocyte globulin are also known to be associated with positive DCT. However, there are cases where a positive Coombs test may be seen even without antibody induced haemolysis like sickle cell disease, renal failure, and multiple myeloma. Hence interpretation of positive DCT should be done keeping in mind complete patient history, clinical data and results of other laboratory investigations.^[9-11]

Very few studies in India have associated positive Coombs test with clinical conditions. The correlation of DCT strength with presence of haemolysis has been reported only in a few studies published in the literature.¹²⁻¹³ In our study we noted that out of 15 cases that tested positive for DCT only 12 were AIHA, 5 negative cases of DCT were AIHA and 1 case of AIHA was ICT positive. Two more DCT positive cases had features of thyroiditis and diabetes mellitus and 1 case was of tuberculosis.

A positive DCT is an important laboratory finding in the diagnosis of AIHA. The clinical and serological features of positive DCT can provide valuable information about the severity and underlying cause of the disease. A comprehensive evaluation of both clinical and serological features is crucial in the management and treatment of AIHA.

Earlier studies have shown DCT positivity in the presence of connective tissue disorders.^[12,13] In a study it was reported that, DCT was positive in 12.8% of SLE patients.^[14] Another author found that the presence of rheumatoid factor (RF) in plasma can lead to both false decrease and false increase in ICT and DCT.^[15] In another study, hepatitis C infection^[16] and tuberculosis^[11] were the common infections showing DCT positivity.^[16] In our study there was one case of tuberculosis which showed DCT positivity. Another study done to evaluate DCT in human immunodeficiency virus (HIV)-infected individuals found many cases with positive coombs test and haemolysis.^[17]

In some studies, the authors saw that the DCT-positive samples were based on the presence or absence of haemolysis and found that 98 (49%) samples showed haemolysis and 102 (51%) samples did not show haemolysis but still were DCT positive.^[2,5] In 5 to 10% of patients with immune haemolytic anaemias, DCT may be negative due to the number of IgG molecules bound to red cells are below the threshold limit of detection or if haemolysis is mediated by IgA antibody.^[5]

In a study on SLE it was seen that a positive DCT in the absence of haemolytic anaemia predicts high disease activity and poor renal response. As mentioned earlier, DCT was positive in 12.8% of SLE studied sample and 54.3% of them had haemolytic anemia.¹⁴ The diagnosis of primary AIHA was established in the remaining 22 of patients. There are reports of DCT positivity and haemolytic anaemia in association with hepatitis C infection.^[16,17]

When interpreting the results of a Direct Coombs test (DCT), there are several patient-related pre-analytical factors that need to be considered. These factors can affect the accuracy of the test results and may lead to false-positive or false-negative results. One such factor is the use of intravenous medications containing colloid solutions. These solutions can cause the red blood cells to form clumps, known as rouleaux formation. This can result in a false-positive DCT result because the antibodies may bind to the clumped cells rather than to the specific antigen on the surface of the red blood cells. In our case the patient of tuberculosis showed false positive DCT positivity.

Another factor to consider is severe haemolysis, which can result in a false-negative DCT result. In cases of severe haemolysis, a large number of red blood cells are destroyed so rapidly that only a few circulating sensitized red blood cells are available for detection by the Coombs test.

Inadequate sample collection or handling can also lead to inaccurate results. A sample that is not properly collected, stored, or transported may lead to haemolysis or other changes in the red blood cells that could affect the test results.

Finally, severe anaemia can also be a pitfall of a negative Coombs test. If the patient's red blood cell count is very low, there may not be enough sensitized red blood cells available for detection, leading to a false-negative result.

It is important to take these patient-related factors into account when interpreting the results of a Coombs test to ensure that the results are accurate and reliable.

CONCLUSION

DCT is the first-line of investigations in patients with haemolysis and in patients with history of transfusion to rule out underlying antibodies to ensure safer transfusion practices. It is important to investigate all DCT-positive samples further, to rule out underlying alloantibodies. A positive DCT must be interpreted in the light of medication history and clinical diagnosis Hence Coombs test forms an important part of investigations for autoimmune haemolysis and should be performed with great precision and care.

Conflict of Interest: All authors have no conflicts of interest

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