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Short communication

Positive direct antiglobulin tests in cancer patients hospitalized with COVID-19: A brief report from India

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1. Introduction

The emergence and rapid global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resulting coronavirus disease 2019 (COVID-19) poses an unprecedented health crisis that was declared as a pandemic by the World Health Organization (WHO) on March 11, 2020. Although, autoimmune haemolytic anaemia (AIHA) is not a common feature of COVID-19 unless patients have an underlying aetiology such as glucose-6-phosphate dehydrogenase deficiency or a sickle cell disorder; a positive direct antiglobulin test (DAT) is frequently observed in patients suffering from COVID-19 [1]. Patients with COVID-19 are at higher risk of thrombosis rather than bleeding but those with severe symptoms may develop anaemia and need blood transfusions [2]. The interpretation of a positive DAT in the context of COVID-19 for transfusion dependent patients such as patients with solid and haematological malignancies is complex. In addition to AIHA, a positive DAT could be found in severe sepsis [3], hypergammaglobulinemia [4], or in association with certain drugs such as antibiotics [5]. Herein we report the incidence and significance of DAT positivity in cancer patients hospitalized with COVID-19.

2. Materials

This was a cross-sectional study during the second and third wave of COVID-19 in India where we studied samples from 88 cancer patients with confirmed COVID-19. The blood samples were sent to the blood centre for pretransfusion testing and DAT. All patients were hospitalized and receiving treatment with multiple drugs (including corticosteroid, antivirals, antibiotics, and vasopressors). No patient had received COVID-19 convalescent plasma and they did not have any positive DAT report in the recent past. Indirect antiglobulin test (IAT) was negative for irregular red cell antibodies in all patients. The consent for testing and transfusion was taken from each patient. However, separate institutional review board approval was not obtained for this study as it was in line with our routine pre-transfusion policy.

Direct antiglobulin test was performed using column agglutination technology (ORTHO BioVue system; Ortho Clinical Diagnostics, Raritan, NJ) with polyspecific antiserum (immunoglobulin G plus complement 3d), and samples that were DAT positive were further investigated using specific antiserum in microcolumn (Ortho Clinical Diagnostics) to differentiate between IgG and complement. If the strength of DAT was 2+ or above with IgG then the acid elution was performed as per the standard operating procedure with an elution kit (BAG- Elutions-Kit, BAG diagnostics, Germany). The eluates were then tested with a commercial three-cell panel of RBCs (Surgiscreen; Ortho Clinical Diagnostics) by performing an IAT to establish whether the IgG antibodies had any RBC antigen specificity. In case the DAT was positive only for C3d, the cold agglutination titration was performed by the conventional tube technique.

A chi-square test was used for discrete variables, and a student *t*-test or a Wilcoxon-Mann-Whitney test was used for continuous variables to look for differences between DAT-positive and DAT-negative patients with *P* values below 0.05 considered as significant. Data were compared unadjusted by a generalized linear model.

3. Results

There was no significant difference between the patients with positive DAT and patients with negative DAT depending on the age, gender, nature of malignancy, and blood group distribution as shown in Table 1. DAT was positive in 32 of 88 (36.4%) patients. Of those 32 patients who were DAT positive, 28 (87.5%) were positive for IgG, one (3.1%) was positive for both IgG and complement, and three (9.4%) were positive for complement. Agglutination was mixed-field in 24 DAT-positive patients (75%), whereas the reaction was more than 2+ only in 6 (18.8%) patients with positive DAT. Out for those six cases two were solely positive for C3d (4+) with a cold agglutination titer value of 2048. None of the eluted samples in rest of the four cases with IgG reactivity showed specificity for RBC

Abbreviations: DAT, Direct antiglobulin test; COVID-19, Coronavirus disease 2019.

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Table 1
Summary of data for COVID-19 patients.

Demography, clinical, and laboratory data	All patients ($n = 88$)	DAT positive patients ($n = 32$)	DAT negative patients ($n = 56$)	P-value
Age, y, median, (range)	63.1 (6.5–80)	62.1 (6.5–80)	64.9 (8–80)	0.2
Male, %	38 (43.18)	13 (40.63)	25 (44.64)	0.12
Blood group, %				
B	50 (56.82)	18 (56.25)	32 (57.14)	0.71
O	19 (21.59)	7 (21.88)	12 (21.43)	0.83
A	15 (17.05)	5 (15.63)	10(17.86)	0.19
AB	4 (4.55)	2 (6.25)	2 (3.57)	0.08
Diagnosis, haematological malignancy, %	40 (45.5)	14 (43.8)	26 (46.5)	0.88
Patients completed COVID-19 vaccination	58 (65.9)	20 (62.5)	38 (67.9)	0.79
Haemoglobin, g/L, median, (95% CI)	89 (85.4–107)	87.5 (82.4–92.6)	99.8 (94.8–112)	0.03
Platelet count, X 10^9/L, median, (95% CI)	80 (77.5–173)	76 (74.7–170.7)	82 (78.7–180.2)	0.11
LDH, U/L, median, (95% CI)	259 (230.8–565.5)	283.5 (255.3–609.4)	258.5 (225.8–550.7)	0.42
Total bilirubin, μmol/L, median, (95% CI)	10.7 (9.8–37.5)	11.1 (10.07–35.07)	10.3 (8.77–39.07)	0.31
Patients receiving red cells transfusion, %	67 (76.14)	25 (78.13)	42 (75)	0.89
Patients on mechanical ventilation, %	25 (28.41)	15 (46.88)	10 (17.85)	0.007
Mortality at 30 d, %[a]	19 (21.59)	13 (40.63)	6 (10.71)	0.002

[a] Mortality at 30 days was calculated from the date of DAT testing.

antigens in the three-cell panel. These results were obtained before patients received blood transfusions for the current admission.

4. Discussion

Direct antiglobulin test is frequently performed at blood centres to diagnose immune-mediated haemolysis. The positivity rate varies from 0.1% in a healthy blood donor population [6] to 1–15% in patients during septic states [7]. Since 2020, several cases have been reported describing the onset of AIHA in COVID-19 patients associated with a positive DAT [8–12]. Although our data indicate that DAT-positive patients had lower haemoglobin concentrations compared to DAT-negative cases but total serum bilirubin and lactate dehydrogenase (LDH) values were not different between two groups which suggest that the anaemia in DAT-positive group was not due to haemolysis rather it could be linked with the severity of the disease. There was no difference in red cells transfusion requirement in DAT-positive patients compared to DAT-negative group. None of the DAT-positive cases demonstrated features of Evans syndrome. The platelet count was similar between two groups; however, the median platelet count was low in the study cohort that might be due to underlying malignancy and effect of chemotherapy.

Underlying mechanisms behind positive DAT among COVID-19 patients are not well understood. Molecular mimicry among SARS-CoV-2 antigens and red cell epitopes seems to be the one mechanism [13]; additionally, the hyper-inflammation triggered by the virus, or excessive complement deposition due to septic state may be other contributing factors [14]. Recently studies have shown that the SARS-CoV-2 infection may cause reduction of RBC membrane deformability, increased membrane protein oxidation, and abnormal membrane lipid composition [15]. It has also been observed that about 10% of patients with critical COVID-19 have neutralising autoantibodies to type I interferons, which is absent in asymptomatic or mild disease [16]. All these factors together might increase the number of RBC exposing phosphatidylserine, and trigger the injury to the red cell membrane. Although there have been cases of acute haemolysis because of cold antibody during SARS-CoV-2 infection [8,11,17] but in our scenario both the patients who had significant cold antibody titer were suffering from diffuse large B-cell lymphoma which is well known to be associated with secondary cold agglutinin syndrome. Therefore, the elevated cold agglutinin titer was not attributed to SARS-CoV-2 infection rather it was due to the B-cell clonal disorder. Majority of patients showed low strength of reactions in the DAT-positive group that might be due to the effect of high-dose steroid treatment given for management of COVID-19 associated pneumonia. The steroid might have shut down the release of immunoglobulins from the B-cell population. There was no difference observed on DAT positivity between the vaccinated and non-vaccinated group but patients who required mechanical ventilation showed a significantly higher DAT positivity rate compared to others. This is similar to the study done by Brochier et al. [18] where the DAT-positivity rate was significantly higher in the ICU patient group (56%) with advanced disease state against those admitted in general wards (35%). Also, the increased mortality rate that was observed in the DAT positive group might be because of more disease severity. A similar DAT result was published by Berzuini et al. [19] who described 46% positivity. They tested elutes with RBCs from DAT negative COVID-19 patients. These results couldnot be reproduced in our experiences, since a different elution kit was used. Elution could be effective as a decision-making tool especially for transfusion dependent patients as suggested by Cabo et al. [20] to identify the presence of underlying alloantibodies. We propose an algorithm in Fig. 1 which could be useful for the blood centres to investigate DAT positive transfusion dependent patients suffering from COVID-19.

The elution was not performed in our study when the strength of DAT was less than 2+. Unfortunately, the criteria for performing elution testing vary across institutions and no systematic method for avoiding uninformative elution testing is available till date [21]. The elution process is a high-burden working process and it is also expensive with respect to labour and reagents. The measurable cost of elution testing amounts to a labour cost in addition to the reagent and other material costs to perform the testing (i.e., technologist wage/hour × hours required for testing + material costs associated with the test). Considering the cost and time required to perform eluate testing, we used more stringent criteria of ≥2+ in this study.

A positive DAT with a negative elution can also be caused by drug-induced antibodies. Since the patients' medication history was complex in the study cohort as most of them were receiving concomitant chemotherapy, this hypothesis cannot be completely ruled out.

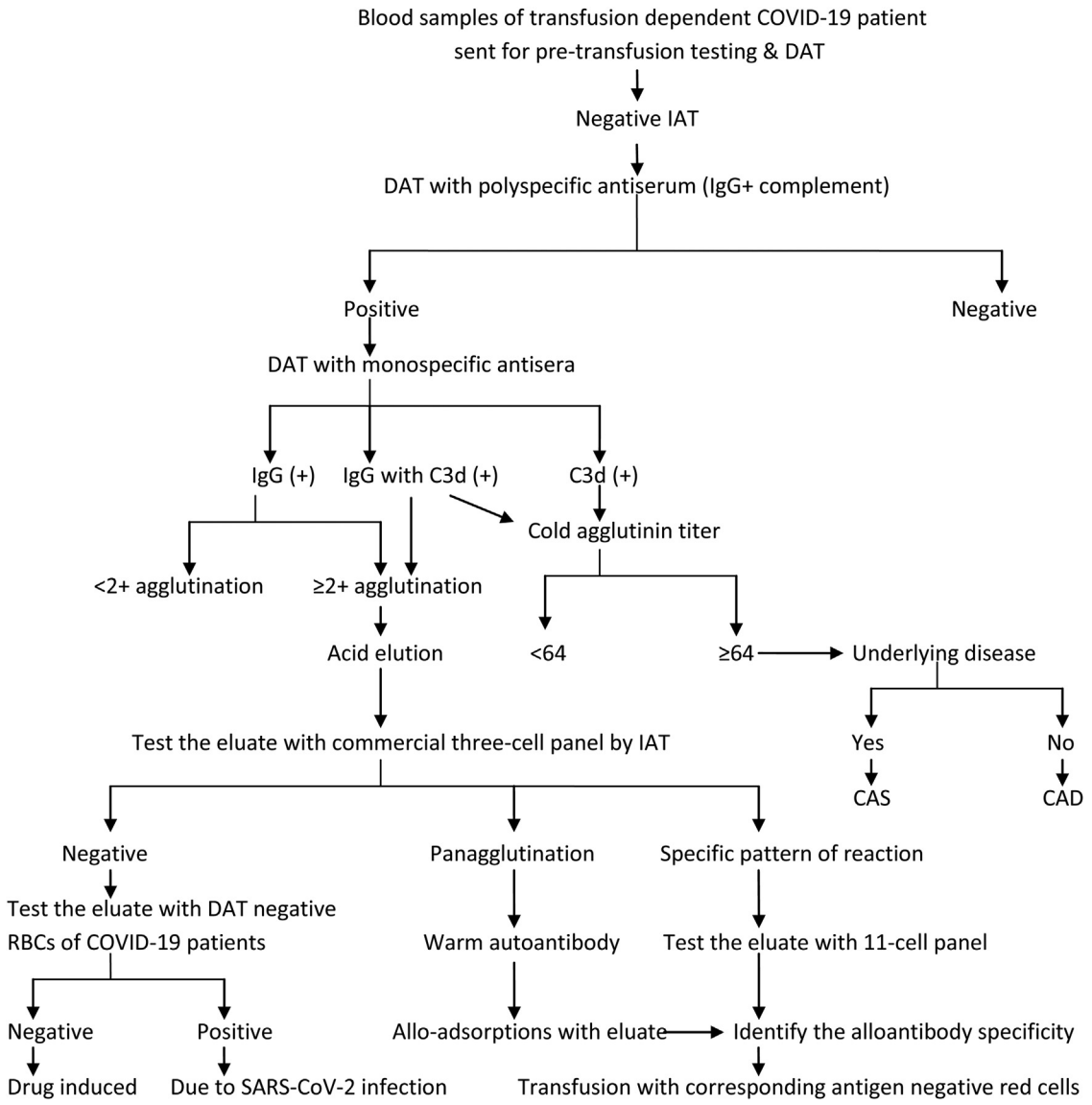

Fig. 1. An algorithm for DAT positive transfusion dependant patients with COVID-19.

5. Conclusion

Results of this study show that a high percentage of cancer patients with COVID-19 are DAT positive, but majority of these patients do not have any evidence of haemolysis and do not require more blood transfusion compared to others. Also, the association of thrombotic complications in COVID-19 patients with DAT positivity needs to be explored further. Finally, the algorithm that triggers the start of the elution process in DAT positive COVID-19 cancer patients who received frequent transfusions should be clearly justified and serum investigation must always be conducted in parallel to avoid unnecessary burden of elution process and extra expenditure.

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Disclosure of interest

The authors declare that they have no competing interest.

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