Positive direct antiglobulin tests in patients with COVID-19

Coronavirus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has now spread globally, affecting more than 7 million people and resulting in over 400 000 deaths. In a recent meta-analysis, there were slight differences in hemoglobin between patients with severe and nonsevere disease (6.52 g/L lower in severe disease) and between survivors and nonsurvivors (1.34 g/L lower in nonsurvivors),[1] but hemolytic anaemia is not a feature of COVID-19 unless patients have developed disseminated intravascular coagulopathy or have an underlying hemolytic anemia such as glucose-6-phosphate dehydrogenase deficiency or a sickle cell disorder. Further, patients with COVID-19 are not at high risk of bleeding, and therefore transfusion requirements for these patients do not appear to be any higher than for patients without COVID-19.[2] Positive direct antiglobulin tests (DATs) due to infections have been reported in pneumonia,[3] hypergammaglobulinemia,[4] and drug-induced immune hemolytic anemia, including in association with antibiotics.[5] In this single large teaching hospital, we investigated the incidence and significance of DAT positivity in patients with COVID-19.

Full blood count samples were collected from 20 consecutive patients in the critical care unit with confirmed SARS-CoV-2 infection confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). On the same day, another 20 consecutive full blood count samples were selected as controls from patients in the critical care unit who were confirmed to be negative for SARS-CoV-2 by RT-PCR on at least two occasions and who had no previous historical positive test. All samples had a DAT performed (BioRad IH-500 analyzer, BioRad Diamed DC-Screening I gels; DiaMed GmbH, Switzerland), and samples that were DAT positive were further investigated using BioRad Diamed DC-Screening II gels and were also eluted with an elution kit (Gamma ELU-KIT II, Immucor, Norcross, Georgia). The eluate was tested against A1 and B cells and a three-cell red blood cell (RBC) screening panel to establish whether the IgG antibodies had any RBC antigen specificity. All washes for the elution were performed inside a microbiological safety cabinet at Containment Level 2.

A χ^2 test was used to look for differences between groups with P values below .05 considered significant.

The median age of the SARS-CoV-2 RT-PCR–positive patients (patients) was 63 years (range, 42-78 years) and 54 years (range, 22-77 years) for the SARS-CoV-2 RT-PCR–negative patients (controls). Ninety-five percent of patients were male, compared to 70% of controls (Table 1). In the patient group, eight (40%) were group O, eight (40%) were group A, three (15%) were group B, and one (5%) was group AB; in the control group, 11 (55%) were blood group O, eight (40%) were blood group A, and one (5%) was blood group B (Table 1). No patient or control had a positive antibody screen.

DAT was positive in 16 of 20 (80%) patients compared with 7 of 20 (35%) controls (significant difference, $\chi^2 = 8.29$; $P = .004$). Of the 16 patients who were DAT positive, all (100%) were positive for IgG, and one (6%) was also positive for C3d. Of the 7 controls who were DAT positive, six (86%) were positive for IgG, and none were positive for C3d. None of the eluted samples in either group showed specificity for RBC antigens in the three-cell screen or against A1 or B cells.

There was no significant difference between the two groups for hemoglobin concentration (patients: median 88 g/L [95% confidence interval, 82-93]; controls: 88 g/L [86-95]; $P = .348$), bilirubin (patients: 6 μmol/L [5-12]; controls: 10 μmol/L [7-15]; $P = .215$) or lactate dehydrogenase (patients: 389 U/L [343-432]; controls: 441 U/L [341-648]; $P = .167$), and no patients or controls had morphologic features of hemolysis.

There was no significant difference between the number of patients (17/20; 85%) or controls (12/20; 60%) who had been on antibiotics within the previous 7 days ($\chi^2 = 3.13$; $P = .077$), nor between the number of DAT-positive samples on those patients and controls who had been on antibiotics (17/29) or those who had not (8/11) ($\chi^2 = 0.68$; $P = .410$).

There was no significant difference in the number of DAT-positive patients who were blood group A or AB (8/16) compared to controls (2/7) ($\chi^2 = 0.910$, $P = .340$), and in the DAT-positive patients 13/16 (81%) had not been transfused, compared to 4/7 (57%) of DAT-positive controls (not a significant difference, $\chi^2 = 1.467$; $P = .226$).

Results of this study show that a high percentage of patients with COVID-19 are DAT positive, and all were positive by IgG, but these patients do not have any evidence of hemolytic anaemia and do not require more blood transfusion than patients who are not infected. No underlying antibody specificity for blood-group antigens was identified in the eluate, and there was no association with antibiotic usage. No patient had a positive antibody screen, and the majority had not received a recent transfusion. These data indicate that DAT-positive results are

TABLE 1 Summary of patient demographics and results for SARS-CoV-2–positive patients

	SARS-CoV-2 positive patients n = 20	SARS-CoV-2 negative controls n = 20
Age, y, median (range)	63 (42-78)	54 (22-77)
Male, %	95	70
Blood group, n (%)		
Group O	8 (40)	11 (55)
O D positive	8	10
O D negative	0	1
Group A	8 (40)	8 (40)
A D positive	8	7
A D negative	0	1
Group B	3 (15)	1 (5)
B D positive	3	1
Group AB	1 (5)	0
AB D positive	1	0
On antibiotics in past 7 days, n (%)*	17 (85)	12 (60)
Hemoglobin, g/L, median (95% CI)	88 (82–93)	88 (86–95)
Bilirubin, μmol/L, median, (95% CI)	6 (5-12)	10 (7-15)
Lactate dehyrdogenase, U/L, median (95% CI)	389 (343-432)	441 (341-648)
Direct antiglobulin test positive, n (%)	16 (80)	7 (35)
IgG positive	15 (94)	6 (86)
IgG and C3d positive	1 (6)	0
IgG and C3d negative	0	1 (6)
Eluate with RBC antigen specificity	0	0

CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

likely to be due to SARS-CoV-2 infection. The pathogenesis of the association is not yet clear but is likely to be multifactorial.

From the clinical transfusion perspective, it is important for laboratories to be aware of this finding, so that for patients who are SARS-CoV-2 positive and DAT positive but have a negative antibody screen and no clinical features of hemolysis, further serologic testing is not required. This will reduce unnecessary staff exposure to infected blood samples.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

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