Letter to the editor: Positive direct antiglobulin tests in patients with COVID-19

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COVID-19, the disease caused by the coronavirus, 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 haemoglobin between patients with severe and non-severe disease (6.52g/L lower in severe disease), and between survivors and non-survivors (1.34g/L lower in non-survivors),[1] but haemolytic anaemia is not a feature of COVID-19, unless patients have developed disseminated intravascular coagulopathy or have an underlying haemolytic anaemia 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 patients without COVID-19.[2] Positive direct antiglobulin tests [DAT] due to infections have been reported in pneumonia,[3] hypergammaglobulinaemia[4] and drug induced immune haemolytic anaemia, 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 RT-PCR. On the same day another 20 consecutive FBC 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 analyser, 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 using the Gamma ELU-KIT II kit (Immucor, Georgia, USA). The eluate was tested against A1- and B-cells and a three-cell red cell screening panel to establish if the IgG antibodies had any red cell 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 0.05 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). 95% 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.

Direct antiglobulin test was positive in 16/20 (80%) of patients compared with 7/20 (35%) of controls (significant difference, χ^2=8.29, p=0.004). Of the 16 patients that 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 red cell antigens in the three-cell screen or against A1 or B cells.

There was no significant difference between the two groups for haemoglobin concentration (patients: median 88 g/L [95% confidence interval 82-93]; controls: 88 g/L [86-95]; p=0.348), bilirubin (patients: 6 µmol/L [5-12]; controls 10 µmol/L [7-15]; p=0.215) or LDH (patients: 389 U/L [343-432]; controls: 441 U/L [341-648]; p=0.167), and no patients or controls had morphological features of haemolysis.

There was no significant difference between the number of patient (17/20 [85%]) or controls (12/20 [60%]) who had been on antibiotics within the previous seven days (χ^2=3.13, p=0.077), 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) (χ^2=0.68, p=0.410).

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) (χ^2=0.910, p=0.340), 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, χ^2=1.467, p=0.226).

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 haemolytic 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 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 SARSCoV-2 positive and DAT-positive, but have a negative antibody screen and no clinical features of haemolysis, further serological testing is not required. This will reduce unnecessary staff exposure to infected blood samples.

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Table 1. Summary of patient demographics and results SARS-CoV-2 positive patients. Median results with 95% confidence intervals except where indicated otherwise

	SARS-CoV-2 positive Patients	SARS-CoV-2 negative Controls
	n=20	n=20
Age, years [median (range)]	63 (42-78)	54 (22-77)
Male [%]	95	70
Blood Group [number (%)]		
Group O	8 (40%)	11 (55%)
O Rh(D) positive	8	10
O Rh(D) negative	0	1
Group A	8 (40%)	8 (40%)
A Rh(D) positive	8	7
A Rh(D) negative	0	1
Group B	3 (15%)	1 (5%)
B Rh(D) positive	3	1
Group AB	1 (5%)	0
AB Rh(D) positive	1	0
On antibiotics in last seven days [number (%)]*	17 (85%)	12 (60%)
Haemoglobin, 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 [number (%)]	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 red cell antigen specificity	0	0