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Research article

Direct antiglobulin test type, red blood cell distribution width, and estimated glomerular filtration rate for early prediction of in-hospital mortality of patients with COVID-19

Fei Chen [a,1], Jing Wang [b,1], Xinhong Jin [a], Bin Li [a], Yili Ying [a], Yufen Zheng [b], Guoguang Lu [b], Jun Li [b,*], Bo Shen [b,*]

[a] Department of Blood Transfusion, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, 150 Ximen Road, Linhai, Taizhou, Zhejiang Province, China
[b] Department of Clinical Laboratory, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, 150 Ximen Road, Linhai, Taizhou, Zhejiang Province, China

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ABSTRACT

Objective: This study aimed to investigate the correlation between COVID-19 and the direct antiglobulin test (DAT) and establish an in-hospital mortality risk predictive model based on the DAT type, which can be used for the early prediction of inpatients with COVID-19.
Methods: In this study, 502 patients admitted to our hospital who underwent DAT testing from January 29 to February 8, 2023, were included (252 DAT-positive and 250 DAT-negative). Among them, 241 cases of COVID-19 were screened(171 DAT-positive and 70 DAT-negative), clinical and laboratory indicators were compared between DAT-positive and DAT-negative groups. Univariate and multivariate logistic regression analysis, the Kaplan-Meier survival curve and receiver operating curves were used to explore the relation between the DAT type and in-hospital mortality of patients with COVID-19.
Results: The proportion of confirmed COVID-19 cases was higher in the DAT-positive group than in the DAT-negative group (67.9 % vs. 28.0 %, $P < 0.05$). Patients with COVID-19 in the DAT-positive group had higher age-adjusted Charlson comorbidity index scores, red blood cell distribution width (RDW), lactate dehydrogenase, prothrombin time, D-dimer, creatinine, and high-sensitive cardiac troponin T levels than the negative group ($P < 0.05$), In contrast, hemoglobin and estimated glomerular filtration rate (eGFR) levels were lower in the DAT-positive group. The DAT-positive group also had a higher red blood cell usage volume and in-hospital mortality rate than the DAT-negative group. The mortality rate of patients with COVID-19 with both IgG and C3d positive was higher than that of the other groups. Multivariate logistic regression analysis showed that RDW and eGFR were associated with mortality in patients with COVID-19. The combined predictive model of DAT type, RDW, and eGFR showed an area under the curve of 0.782, sensitivity of 0.769, and specificity of 0.712 in predicting in-hospital mortality risk in patients with COVID-19.
Conclusion: The established predictive model for in-hospital mortality risk of patients with COVID-19 based on DAT type, RDW, and eGFR can provide a basis for timely intervention to reduce the mortality rates of patients with COVID-19. This model is accessible at https://jijijiduola.shinyapps.io/0531// for research purposes.

1. Introduction

During the Omicron outbreak, the Department of Clinical Blood Transfusion work has faced significant challenges. Owing to increased direct antiglobulin test (DAT) positivity in patients with COVID-19 with severe anemia, it was increasingly challenging to find compatible blood units. We found that among the severe and critical patients with COVID-19 who require a blood transfusion, the positive rate of DAT is as high as 90 %, consistent with that in patients with autoimmune hemolytic anemia reported by previous studies[1,2].

* Corresponding authors.
E-mail addresses: lij@enzemed.com (J. Li), shenb@enzemed.com (B. Shen).
[1] These authors contributed equally to this work.

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The anti-human globulin test is used to detect the presence of antibodies or complement components in the serum or on red cell membranes and was first used in clinical medicine by Robin Coombs in 1945 [3]. DAT uses an anti-human globulin reagent to form visible agglutination reactions, which are used to verify whether red blood cells are sensitized with antibodies and/or complement components in the patient's body and the intensity of agglutination and the number of bindings are directly proportional[4]. Based on the pattern of the patient's red blood cells reacting with the anti-human globulin reagent, DAT-positive cells can be further divided into IgG, C3d, and IgG+C3d types. DAT is often used to confirm autoimmune hemolytic anemia (AIHA), a rare and complex disease with diverse clinical manifestations and variable severity[5]. Case reports have indicated that DAT positivity is common in patients with Mycoplasma pneumonia, EB virus infection virus infection, influenza virus infection, and COVID-19[6–8]. Excessive activation of the complement system can lead to red blood cell lysis and exacerbate hemolytic anemia. Studies have shown that patients with COVID-19 with underlying diseases may develop severe AIHA, resulting in patients requiring blood transfusion, exchange transfusion, multiple organ failure, or even death[9].

In previous literature, other pathogens have been excluded from the reported cases, supporting an association between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and secondary AIHA. Almost all patients in these studies showed positive DAT with IgG, C3d, and IgG+C3d antibodies. These studies also indicate a high prevalence of DAT positivity in patients with COVID-19, confirming that cases of hemolysis tend to present with IgG+C3d or C_3d[10,11]. However, the mechanism underlying DAT positivity in patients with COVID-19 remains unclear.

In this study, we aim to examine the correlation between DAT type and the prognosis of patients with COVID-19 analyze the factors contributing to mortality, and also develop a predictive model for COVID-19 mortality based on the DAT typing results and other indicators for early clinical intervention and offer new laboratory evidence for diagnosing and treating patients with COVID-19.

2. Materials and methods

2.1. Cohort selection

We collected data from 536 hospitalized patients who underwent DAT testing (anti-IgG+C3d, anti-IgG, and anti-C3d) between January 29 and February 8, 2023. The following patients were excluded: 1) patients with a history of autoimmune hemolytic anemia (n = 5), 2) patients with a history of blood transfusion before admission (n = 5), and 3) patients with a history of blood transfusion before the DAT (24). Finally, 502 patients were included in the study (Fig. 1). All patients' data were collected, collated independently, and finally reviewed. The study was approved by the Ethics Committee of Taizhou Hospital, Zhejiang Province (Ethics approval number: 20230116).

2.2. Direct antiglobulin test

Changchun Boxun (China) anti-human globulin test cards (anti-IgG+C3d, anti-IgG and anti-C3d), and a TD-A medical centrifuge were used for the direct anti-human globulin test in this study. The red blood cells were washed three times with 0.9 % sodium chloride solution and prepared into a 0.8 % red blood cell suspension, which was added to an anti-human globulin test card (anti-IgG+C3d, anti-IgG, anti-C3d, and control). Then, 50 μL per well was added and centrifuged for 5 min (900g for 2 min and 1500g for 3 min). Positive effect of an anti-human globulin test card (anti-IgG+C3d) indicates the existence of IgG antibodies on the patient's red blood cells and/or sensitization by C3d complement; a positive result of an anti-IgG well suggests the presence of IgG antibody; a consequence of an anti-C3d well indicates the sensitization by C3d complement. The control well was the negative control, and if there was a positive result, the result was questionable, and the test needed to be repeated. If the results of the three wells were inconsistent, the test was repeated using the classic anti-human globulin test.

2.3. COVID-19 diagnostic criteria

According to the Chinese COVID-19 Diagnosis and Treatment Protocol (10th edition), the diagnostic criteria included clinical

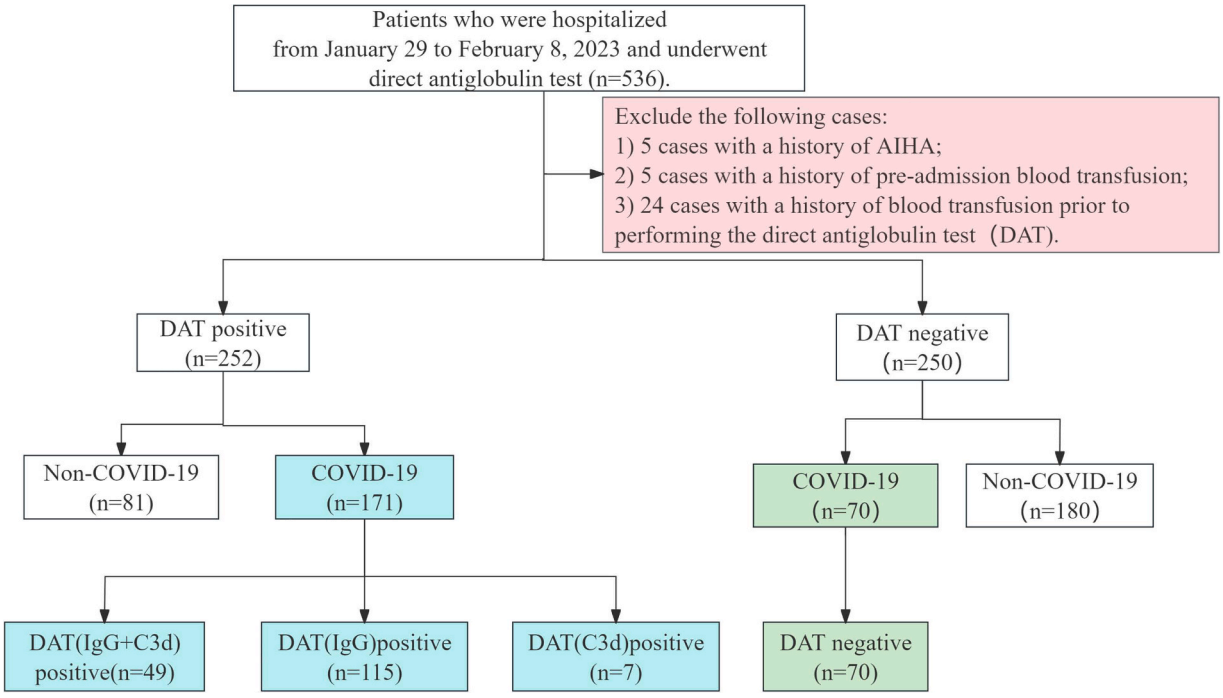


Fig. 1. The flow chart of the cohorts.

manifestations, chest computed tomography (CT), and etiological examination. RT-PCR of nasopharyngeal swabs was used to diagnose patients with COVID-19 and conventional viral pneumonia.

2.4. Data collection

The clinical data of 502 patients were collected from electronic medical records, including sex, age, underlying disease, medical history, vaccination history, onset time of COVID-19 symptoms, medication history, chest CT data, red blood cell utilization, hospital stay, and treatment outcomes. Relevant laboratory data were collected, including routine blood data (hemoglobin, red blood cell distribution width, and reticulocytes) on the day of the DAT test, biochemical indicators (total bilirubin, alanine aminotransferase, lactate dehydrogenase, creatinine, glomerular filtration rate, high-sensitivity troponin, and oxygenation index), and coagulation indicators (prothrombin time, D-dimer, and partial prothrombin) within three days before and after the DAT test.

2.5. Statistical analysis

SPSS (version 23.0), GraphPad Prism (version 8.0), and R software (version 3.5.1) were used for statistical analysis. Numbers and percentages represented the categorical variables, the continuous variables with normal distribution were described by mean and standard deviation (mean ± SD), and the continuous variables with non-normal distribution were represented by median or quartile (interquartile range). Differences among groups were compared using the chi-square, t-test, and Mann-Whitney U tests. A multivariate logistic regression model was used to determine factors affecting the mortality of patients with COVID-19. The Kaplan-Meier survival curve was used to compare the effects of different DAT types on the survival of patients with COVID-19. The receiver operating characteristic curve was used to analyze the predictive value of DAT in combination with red blood cell distribution width (RDW) and estimated glomerular filtration rate (eGFR) for the risk of mortality from COVID-19.

3. Results

3.1. Clinical information of study cohorts

Of the 502 patients included in this study, the average age was 69 years, and males accounted for 68.3 %. The primary diagnoses were pneumonia (266 patients), malignant lung tumors (72 patients), obstructive pulmonary disease (33 patients), acute cerebral hemorrhage or infarction (25 patients), pulmonary embolism (14 patients), coronary atherosclerotic heart disease (10 patients), and other diseases (82 patients). Among them, 252 DAT-positive and 250 DAT-negative cases were identified using the microcolumn gel anti-human globulin test. There was no significant difference in sex between the two groups ($P > 0.05$), but there were significant differences in age, age-adjusted Charlson comorbidity index (ACCI) scores, and pneumonia as the principal diagnoses on admission between the two groups ($P < 0.05$). PCR testing confirmed 241 cases of COVID-19 and 261 non-COVID-19 cases. The DAT positivity rates were 71.0 % for COVID-19 cases and 31.0 % for non-COVID-19 cases, showing a significant difference ($P < 0.001$). Mortality in the DAT-positive COVID-19 group (21.6 %) was significantly higher than that in the DAT-negative COVID-19 group (8.6 %, $P = 0.016$), DAT-positive non-COVID-19 group (7.4 %, $P = 0.005$), and DAT-negative non-COVID-19 group (4.4 %, $P < 0.001$). No significant differences were observed among the three groups. Further details are provided in Table 1 and Fig. S1.

Table 1
Clinical information of study cohorts(n = 502).

	DAT positive (n = 252)	DAT negative (n = 250)	*P value*
Age, years, median (IQR)	76(67-84)	67(56-77)	< 0.001
Sex, No(%)			
Male	179(71.0)	164(65.6)	0.225
Female	73(29.0)	86(34.4)	
ACCI score, No(%)			
≤ 2	41(16.3)	75(30.0)	< 0.001
3-5	117(46.4)	126(50.4)	
> 5	94(37.3)	49(19.6)	
Main diagnosis on admission, No (%)			
Pneumonia	165 (65.5)	101 (40.4)	< 0.001
pulmonary malignant tumor	26 (10.3)	46 (18.4)	0.011
Obstructive pulmonary disease	9 (3.6)	24 (9.6)	0.007
other	52 (20.6)	79 (31.6)	0.006
COVID-19, No(%)	171 (67.9)	70 (28.0)	< 0.001
non-COVID-19, No (%)	81 (32.1)	180 (72.0)	< 0.001

Note: The bold values were $P < 0.05$.
Abbreviations: ACCI, Age-Adjusted Charlson Comorbidity Index.

3.2. Analysis of DAT results and related factors in patients with COVID-19

We analyzed the factors associated with DAT results based on a further study of 241 confirmed COVID-19 cases (Table 2). For comparison, patients with COVID-19 were tested for DAT and divided into DAT-positive and DAT-negative groups. The comparison showed no statistically significant differences between the two groups regarding age, sex, medication history, vaccination history, vaccine type, vaccination time, COVID-19 clinical classification, or pulmonary CT. Additionally, there was no statistically significant difference in the interval between specimen testing time and symptom onset.

In patients with COVID-19, the ACCI score ($P < 0.001$) was significantly higher in DAT-positive patients than in the DAT-negative group, whereas in patients with non-COVID-19, there was no statistical difference between DAT-positive and DAT-negative groups ($P = 0.528$). In this study, the hematology indexes within three days of the DAT detection time were counted, and no statistical significance was found in the reticulocyte, bilirubin, partial prothrombin, alanine aminotransferase, and oxygenation indices. Hemoglobin (Hb) ($P = 0.004$), RDW ($P = 0.036$), lactate dehydrogenase ($P = 0.022$), prothrombin time ($P = 0.001$), D-dimer ($p = 0.036$), creatinine ($P = 0.001$), eGFR ($P < 0.001$), and high-sensitive cardiac troponin T ($P = 0.001$) were significantly different.

3.3. The impact of different DAT typing results on clinical and laboratory indicators in patients with COVID-19

One hundred and seventy-one DAT-positive patients were divided into IgG+C3d, IgG, and C3d groups according to the reaction pattern of red blood cells and the anti-human globulin reagent, including 115 cases of IgG (47.7 %), seven cases of C3d (2.9 %), and 49 cases of IgG+C3d (20.3 %) (Fig. 2A).

The indicators with statistical differences in Table 2 were further compared among the subgroups. The results showed that Hb and RDW were statistically different between the DAT-negative and IgG+C3d groups, but there was no statistically significant difference between the DAT-negative and IgG groups. In contrast, lactate dehydrogenase differed only between the DAT-negative and IgG groups (Fig. 2B–J).

3.4. Comparison of blood transfusion requirements and in-hospital mortality among different DAT types in patients with COVID-19

We followed up on the data on the blood requirements of patients with COVID-19 during their hospitalization after the DAT. In the DAT-positive group, 21 patients received at least one red blood cell transfusion (146 U), whereas no transfusion cases were found in the DAT-negative group. The groups showed significant differences in the

Table 2
Analysis of factors associated with DAT in COVID-19 patients.

Variables	DAT positive (n = 171)	DAT-negative (n = 70)	*P value*
Age, years, median (IQR)	77(68-84)	75(62-82)	0.052
Sex, No. (%)			
Male	125(73.1 %)	43(61.4 %)	0.073
Female	46(26.9 %)	27(38.6 %)	
ACCI score, No. (%)			
≤ 2	14(8.2)	18(25.8)	**< 0.001**
3-5	80(46.8)	32(45.8)	
> 5	77(45.0)	20(28.6)	
COVID-19 clinical type			
Mild&medium	40(23.4)	25(35.7)	0.065
severe	88(51.5)	35(50.0)	
critically severe	43(25.1)	10(14.3)	
Interval from COVID-19 onset to DAT collection, days, No. (%)			
0∼≤ 7	9(5.3)	8(11.4)	0.310
> 7∼≤ 14	12(7.0)	5(7.1)	
> 14∼≤ 21	24(14.0)	5(7.1)	
> 21∼≤ 28	45(26.3)	17(24.3)	
> 28	81(47.4)	35(50.0)	
Treatment, No. (%)			
Antibiotic	169(98.8)	68(97.1)	0.352
NSAID	16(9.4)	5(7.1)	0.580
Antiviral drug	20(11.7)	12(17.1)	0.428
Methylprednisolone	83(48.5)	46(65.7)	0.072
Immunoglobulin	30(17.5)	9(12.9)	0.370
Anticoagulants	38(22.2 %)	18(25.7 %)	0.560
COVID-19 Vaccination History, No. (%)			
yes	100(58.5)	46(65.7)	0.283
no	31(18.1)	7(10.0)	
unknown	40(23.4)	17(24.3)	
Vaccine type, No. (%)			
Inactivated vaccine	60(59.4)	30(66.0)	0.707
Hybrid immunity	19(19.8)	9(19.1)	
other	21(20.8)	7(14.9)	
Symptom onset to last vaccination time, months, No. (%)			
> 12	24(25.5)	13(27.7)	0.856
6-12	43(42.5)	19(42.6)	
< 6	33(32.1)	14(29.8)	
Inspection indicators			
Hb, g/L, mean±SD	99 ± 24	108 ± 19	**0.004**
RDW, %, mean±SD	14.9 ± 2.2	14.3 ± 1.8	**0.036**
RET, %, mean±SD	2.5 ± 1.8	2.2 ± 1.3	0.260
LDH, U/L, median (IQR), n = 73	315(224-461)	245 (204-255)	**0.022**
TBIL, μmol/L, mean±SD	13.7 ± 13.1	10.7 ± 4.0	0.071
PT, s, median (IQR), n = 129	14.4(13.7- 16.1)	13.6(12.8- 14.4)	**0.001**
APTT, median (IQR), n = 129	40.3(35.4- 46.0)	38.9(36.2-42.7)	0.471
D-dimer, mg/L, median (IQR), n = 163	1.57(0.92-2.73)	1.05 (0.80-1.85)	**0.036**
ALT, U/L, mean±SD	44 ± 101	41 ± 43	0.812
Cr, μmol/L, mean±SD	104 ± 85	68.± 23	**0.001**
eGFR, mL/min, mean±SD	71 ± 30	88 ± 21	**< 0.001**
hs-cTnT, g/L, median (IQR), n = 113	0.04 (0.02- 0.07)	0.01(0.01-0.03)	**0.001**
OI, mmHg, mean±SD	282 ± 118	294 ± 124	0.545
CT manifestations, No. (%)			
Normal	1(0.6)	1(1.3)	0.512
Multiple patchy opacities and interstitial changes	110(64.7)	52(72.0)	0.153
Multiple ground-glass and infiltrative opacities	41(24.1)	13(21.3)	0.361
Pulmonary consolidation	19(11.2)	4(5.3)	0.195

Note: The bold values were $P < 0.05$.
Abbreviations: ACCI, age-adjusted Charls on Comorbidity Index; Hb, hemoglobin; RDW, red cell distribution width; RET, reticulocyte; LDH, lactate dehydrogenase; TBIL, total bilirubin; PT, prothrombin time; APTT, activated Partial Thromboplastin Time; ALT, alanine aminotransferase; Cr, creatinine; eGFR, estimated glomerular filtration rate; hs-cTnT, high-sensitivity cardiac troponin T; OI, oxygenation Index.
Figure legends

number of patients receiving transfusions ($P = 0.002$) and blood transfusion volume ($P = 0.021$), and the transfusion volume of red blood cells in IgG+C3d was significantly higher than that in the other DAT types (Fig. 3A).

The inpatient mortality rate of patients with COVID-19 between the DAT-positive and DAT-negative groups differed ($P = 0.040$). The in-hospital mortality rates of IgG+C3d, IgG, C3d, and DAT-negative groups were 30.6 %(15/49), 14.8 %(17/115), 14.3 %(1/7), and 8.6 %(6/70), respectively, and there was a significant difference ($P = 0.020$). The mortality rate in the IgG+C3d group was higher than in the other groups (Fig. 3B).

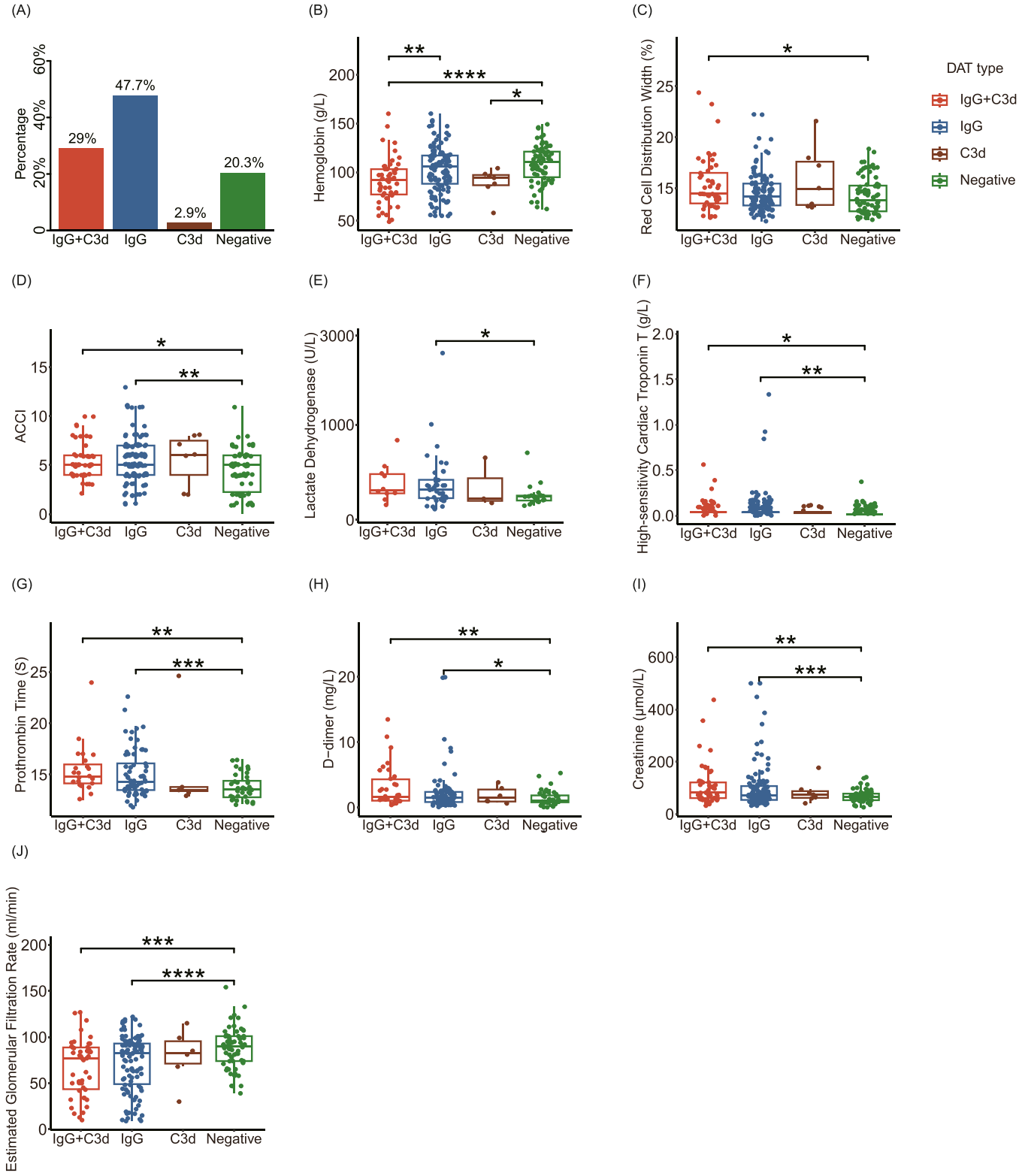


Fig. 2. Comparison of the proportion and clinical and laboratory indicators of DAT subgroups. A.The proportion of different results of DAT in patients with COVID-19; B–J Boxplots of ACCI, hemoglobin, red cell distribution width, estimated glomerular filtration rate, creatinine, prothrombin time, activated partial thromboplastin time, and high-sensitivity cardiac troponin T between IgG+C3d, IgG, C3d, and DAT-negative COVID-19 patients. Note: * , $0.01 < P < 0.05$; * *, $0.005 < P < 0.01$; * ** , $0.001 < P < 0.005$; * ** *, $P < 0.001$.

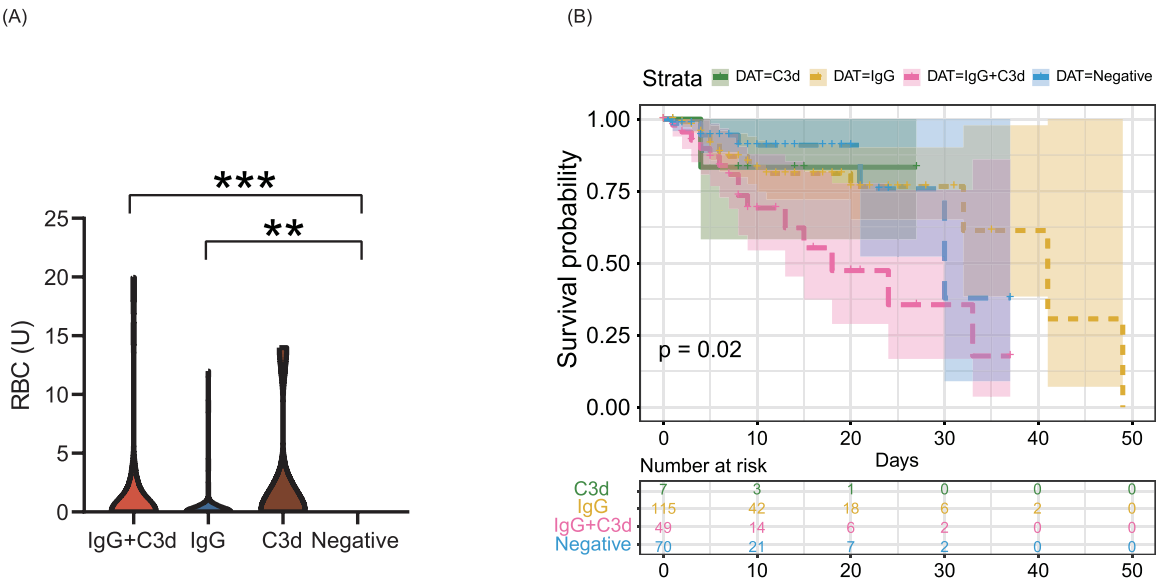


Fig. 3. Comparison of blood transfusion requirements and in-hospital mortality of DAT subgroups. A. Boxplots of transfusion requirements among hospitalized DAT-negative, IgG, IgG+C3d, and C_3d COVID-19 patients; B. Relationship between DAT results and survival risk in patients with COVID-19.

3.5. The relationship between clinical and laboratory indicators and the survival outcome of patients with COVID-19

The sensitivity and specificity of using negative or positive DAT results to predict the in-hospital mortality of patients with COVID-19 were 0.846 and 0.321, respectively. When different types of DAT were used, the sensitivity and specificity were 0.385 and 0.832, respectively. We aimed to identify indicators with differences between DAT-positive and DAT-negative groups to supplement DAT for the early prediction of in-hospital mortality in patients with COVID-19. Univariate and multivariate logistic regression analyses showed that RDW and eGFR were associated with in-hospital mortality in patients with COVID-19 (Fig. 4A).

3.6. Early prediction of mortality in patients with COVID-19 using DAT Typing results, RDW, and eGFR

The area under the curve of the qualitative DAT results, DAT typing results, RDW, and eGFR for mortality prediction in patients with COVID-19 were 0.583, 0.643, 0.725, and 0.701, respectively. DAT typing results combined with RDW and eGFR had an area under the curve of 0.782, sensitivity of 0.769, and specificity of 0.712, using a cutoff value of 0.149. Patients with COVID-19 had a lower risk of death when they were DAT-negative, RDW < 13.95 %, and eGFR > 51. The predictive model for mortality was: $p(Y=1|X)= 1/\{1 +\exp[-(-5.415 +0.272 \times DAT\_type+0.344 \times RDW-0.024 \times eGFR)]\}$ (Fig. 4B).

4. Discussion

COVID-19 treatment usually includes anti-infection, oxygen therapy, anticoagulation, and underlying disease support. However, little attention has been paid to AIHA in the early stages of treatment, which is secondary to SARS-CoV-2 infection. This study found that in patients with COVID-19, the positive rate of the DAT was significantly increased. Compared with DAT-negative patients, the blood demand and hospital mortality of DAT-positive patients were higher, and IgG+C3d-positive patients had higher blood demand and in-hospital mortality than other types. In this study, we established a mortality prediction model based on DAT typing results combined with the RDW and eGFR of patients with COVID-19 with high accuracy, which can provide a reference value for early clinical intervention.

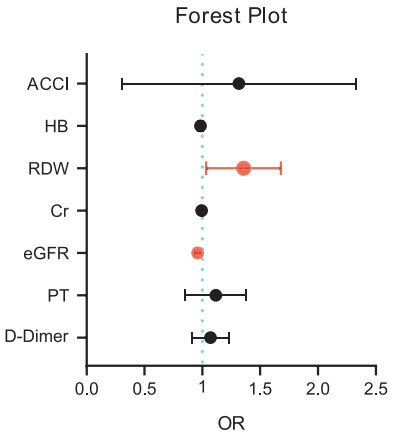
In our cohort, 50.2 % of the patients were DAT-positive. Among the patients diagnosed with COVID-19, the positive rate of DAT was 71.0 %, and that of non-COVID-19 patients was 31.0 %. In patients with COVID-19, the ACCI was positively correlated with the DAT-positive rate, and there was no statistical difference in non-COVID-19 cases. Only 0.1 % of healthy blood donors were reported to be DAT-positive, and the incidence of DAT positivity in hospitalized patients generally ranges from 1 % to 15 %[12]. This study found that the incidence of DAT positivity in hospitalized patients was much higher than that reported in other studies, which might be because this study was conducted during the Omicron outbreak. The enrolled patients consisted of 241 patients (48.0 %) with confirmed COVID-19, 137 (27.3 %) with a history of SARS-CoV-2 infection, and 124 (24.7 %) uninfected individuals. Therefore, we speculate that the COVID-19 infection may contribute to an increase in the DAT-positive rate. Long et al.[13] found a significantly higher DAT-positive rate (46 %) in patients with COVID-19 than in non-COVID-19 transfusion applicants (10 %), supporting the findings of this study.

In this study, 67.2 % of the DAT-positive COVID-19 patients were IgG-positive alone, 28.7 % were IgG+C3d positive, and 4.1 % were C3d-positive alone. Matsuura et al.[14] reported that of 18 patients with DAT-positive COVID-19, 11 (61 %) were IgG-positive, 7 (39 %) were IgG+C3d-positive, and none were C3d-positive. In addition, Berzuini et al.[15] found that of 113 patients infected with SARS-CoV-2, 46 % were DAT-positive, 88 % were IgG-positive, 8 % were IgG+C3d-positive, and 4 % were C3d-positive. The difference in the proportion of DAT types in this study and other studies may be related to the sample size.

We further analyzed the effect of DAT positivity on patient blood requirements. Patients with a history of blood transfusion before DAT testing were excluded from this study. Among the tracked patients with COVID-19, 21 individuals in the DAT-positive group received at least one time of red blood cell transfusion during hospitalization, totaling 146 units (1 U for 200 mL of cells isolated from whole blood). No transfusions were performed in the DAT-negative group. Additionally, patients with IgG+C3d positivity were more likely to receive blood transfusions. During the Omicron outbreak, when blood supply was severely limited, blood distribution was strictly following the AIHA treatment guidelines, suggesting that transfusions should be avoided or minimized and that it is appropriate to transfuse when hemoglobin levels are between 50–70 g/L if symptoms of intolerance occur;

(A)

Variables	Univariate Analysis OR (95%CI)	P value	Multivariate Analysis OR (95%CI)	P value
ACCI	1.837(1.056-3.197)	0.031	1.056(0.459-2.433)	0.898
HB	0.959(0.942-0.976)	0.000	0.984(0.961-1.008)	0.186
RDW	1.382(1.187-1.608)	0.000	1.331(1.047-1.692)	0.019
Cr	1.006(1.002-1.010)	0.002	0.994(0.985-1.003)	0.218
eGFR	0.974(0.962-0.986)	0.000	0.959(0.93-0.989)	0.007
PT	1.275(1.072-1.518)	0.006	1.095(0.862-1.391)	0.457
D-Dimer	1.257(1.086-1.455)	0.002	1.062(0.915-1.234)	0.430
LDH	1.002(0.999-1.004)	0.148		
hscTNT	1.267(0.535-3.000)	0.590		



(B)

predictive indicators	AUC	P	Sensitivity	Specificity
DAT_type	0.643	0.004	0.385	0.832
DAT	0.583	0.078	0.846	0.321
RDW	0.725	0.000	0.872	0.522
eGFR	0.701	0.000	0.538	0.853
DAT_type#RDW#eGFR	0.782	0.000	0.769	0.712

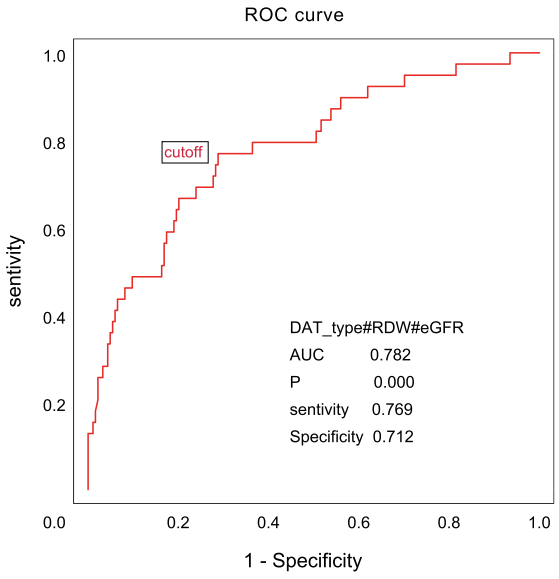


Fig. 4. The screening and predictive effectiveness of risk factors. A. Association of clinical and laboratory indicators and survival outcomes in patients with COVID-19; B. Comparison of ROC analysis between single and combined parameters for predicting mortality risk in patients with COVID-19. The ROC curve was used to predict the mortality rate by combining DAT type, RDW, and eGFR.

transfusion is recommended when hemoglobin levels fall below 50 g/L. Red blood cell transfusions were administered with identical ABO and RhD blood types. Units with the least reactivity were selected for transfusion in cases of incomplete cross-match compatibility. No severe adverse reactions to transfusion were observed.

In addition, this study showed that the mortality rate in the DAT-positive group was significantly higher than that in the DAT-negative group of patients with COVID-19 and in the DAT-positive and DAT-negative groups of non-COVID-19 patients. Furthermore, among patients with COVID-19, there were significant differences in mortality risks associated with different DAT-type results, with the DAT (IgG+C3d)-positive group exhibiting a notably higher risk than the other groups. When a patient is infected with SARS-CoV-2, the immune system may erroneously recognize ankyrin 1protein on the erythrocyte membrane as the spike (S) protein epitope of SARS-CoV-2[16]. This misrecognition may result in an autoimmune response of the immune system against ankyrin one protein, leading to red blood cell damage and hemolytic anemia. Diao et al.[17] reported that SARS-CoV-2 directly infects renal tubules and causes acute renal tubular injury. Immune complexes filter through the glomeruli and are deposited in the renal tubules, causing tubular damage and acute kidney injury. Mechanisms such as the overactivation of the complement system, endothelial dysfunction, and consumption of coagulation factors affect the coagulation system, leading to an increased risk of thrombosis and bleeding [18,19]. Free hemoglobin activates the complement system, which strikes the endothelium and the clotting system[20]. In this study, the Hb and eGFR levels of the IgG+C3d positive group were significantly lower than those of the other groups, and the RDW, creatinine, and D-dimer levels differed from those of the different groups. Therefore, we speculate that positive DAT results, especially those for IgG and C3d, are more likely to lead to severe anemia and organ dysfunction, such as in the lungs and kidneys, thereby increasing the risk of death.

The clinical symptoms of AIHA are heterogeneous, and early diagnosis is difficult. Some blood indicators, such as RDW, have relatively high sensitivity but poor specificity. Furthermore, hemolysis does not necessarily occur even if the DAT is positive. Although DAT results can

guide the differential diagnosis of AIHA, they are usually performed after confirming the presence of hemolysis[21]. Severe COVID-19 is often recognized after severe hemolytic reactions and organ damage have occurred, resulting in irreversible damage to patients owing to delayed treatment. The mortality rate in severe cases is approximately 50 %[22].

The strength of this study is that after applying strict exclusion criteria for included cases, an early death prediction model for patients with COVID-19 was established based on DAT typing results, together with RDW and eGFR. This model is convenient and has high sensitivity, specificity, and clinical application value. Among the deceased patients in this study cohort, 28 could be identified early if the model was used for early screening and early and effective interventions, such as glucocorticoids, rituximab, immunoglobulin injection or plasma exchange, and blockade with complement inhibitors[21,23], may be effective in reducing disease progression and even death in patients. In our cohort, 36 cases met the criteria with an estimated mortality risk ≥ 0.149, ACCI > 5, and COVID-19 severity of severe or critical. Excluding one death case without glucocorticoid use, a comparison of 19 survival cases to 16 deaths revealed earlier glucocorticoid initiation in the survival group ($P = 0.035$), suggesting that early intervention may reduce mortality in critically ill patients, which is supported by other literatures [24–26].

This study had certain limitations. First, this was a retrospective study, and there was insufficient uniformity in the timing of DAT testing and no continuous follow-up of the patients' DAT results. Second, almost all enrolled patients were treated with antibiotics, and whether antibiotics contribute to DAT positivity remains uncertain. In addition, factors such as prolonged bed rest, malnutrition, fasting, mobility impairment, dehydration, and recent hemodialysis in certain patients may challenge the reliability of eGFR as an accurate measure of renal function. Our team will further investigate the autoimmune mechanisms of SARS-CoV-2 and evaluate the applicability of this model to severe pneumonia caused by other viruses.

5. Conclusion

In this study, we established a mortality prediction model for patients with COVID-19 based on DAT classification results combined with RDW and eGFR indicators, which can sensitively identify high-risk patients early in the disease. We suggest that patients with COVID-19 monitor their DAT results and that DAT-positive patients should be further classified. For patients with IgG+C3d-positive, RDW ≥ 13.95 %, eGFR ≤ 51, and combination index ≥ 0.149, close attention should be paid to patient disease changes, and timely clinical intervention should be conducted to reduce mortality.

Ethical Approval Information

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Medical Ethics Review Board of Taizhou Hospital of Zhejiang Province (No: K20230116; date of approval: 23 January 2023).

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CRediT authorship contribution statement

Fei Chen: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Jing Wang:** Data curation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Xinhong Jin:** Data curation, Formal analysis, Software, Visualization. **Bin Li:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Validation. **Jun Li:** Conceptualization, Investigation, Methodology, Project administration, Supervision. **Bo Shen:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision. **Yili Ying:** Investigation, Resources, Validation. **Yufen Zheng:** Conceptualization, Formal analysis, Methodology. **Guoguang Lu:** Resources, Validation.

Declaration of Competing Interest

Authors state no conflict of interest.

Data Availability

The data presented in this study are available on request from the corresponding author.

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Not Applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.07.002.

References

[1] Kamesaki T, Toyotsuji T, Kajii E. Characterization of direct antiglobulin test-negative autoimmune hemolytic anemia: a study of 154 cases. Am J Hematol 2013;88(2):93–6. https://doi.org/10.1002/ajh.23356.
[2] Chaudhary R, Das SS, Gupta R, Khetan D. Application of flow cytometry in detection of red-cell-bound IgG in coombs-negative AIHA. Hematology 2006;11(4):295–300. https://doi.org/10.1080/10245330600915958.
[3] Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and "incomplete" RH agglutinins. Br J Exp Pathol 1945;26(4):255–66.
[4] Parker V, Tormey CA. The direct antiglobulin test: indications, interpretation, and pitfalls. Arch Pathol Lab Med 2017;141(2):305–10. https://doi.org/10.5858/arpa.2015-0444-RS.
[5] Rodberg K. DAT-negative autoimmune hemolytic anemia. Hematol Oncol Clin North Am 2022;36(2):307–13. https://doi.org/10.1016/j.hoc.2021.11.004.
[6] Stein B, DeCredico N, Hillman L. Evaluation of the direct antiglobulin test (DAT) in the setting of mycoplasma pneumoniae infection. JAMA 2018;319(13):1377. https://doi.org/10.1001/jama.2018.1969.
[7] Horwitz CA, Moulds J, Henle W, Henle G, Polesky H, et al. Cold agglutinins in infectious mononucleosis and heterophil-antibody-negative mononucleosis-like syndromes. Blood 1977;50(2):195–202. https://doi.org/10.1182/blood.V50.2.195.195.
[8] Zagorski E, Pawar T, Rahimian S, Forman D. Cold agglutinin autoimmune haemolytic anaemia associated with novel coronavirus (COVID-19). Br J Haematol 2020;190(4):e183–4. https://doi.org/10.1111/bjh.16892.
[9] Jacobs J, Eichbaum Q. COVID-19 associated with severe autoimmune hemolytic anemia. Transfusion 2021;61(2):635–40. https://doi.org/10.1111/trf.16226.
[10] Cabo J, Brochier A, Saussoy P, Van Dievoet M-A, Capirchio L, et al. Positive direct antiglobulin test in COVID-19 patients: decision-making process. Transfus Clin Et Biol 2021;28(4):414–9. https://doi.org/10.1016/j.tracli.2021.05.010.
[11] Wahlster L, Weichert-Leahey N, Trissal M, Grace RF, Sankaran VG. COVID-19 presenting with autoimmune hemolytic anemia in the setting of underlying immune dysregulation. Pedia Blood Cancer 2020;67(9):e28382. https://doi.org/10.1002/pbc.28382.
[12] Shah S, Padrnos L. Complications of autoimmune hemolytic anemia. Hematol/Oncol Clin North Am 2022;36(2):353–63. https://doi.org/10.1016/j.hoc.2021.12.003.
[13] Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26(6):845–8. https://doi.org/10.1038/s41591-020-0897-1.
[14] Matsuura H, Fujii S, Matsui Y, Sugiura Y, Akiyama H, et al. An association between a positive direct antiglobulin test and HLA-DR12 in COVID-19. Ann Hematol 2022;101(9):1959–69. https://doi.org/10.1007/s00277-022-04921-9.
[15] Berzuini A, Bianco C, Paccapelo C, Bertolini F, Gregato G, et al. Red cell–bound antibodies and transfusion requirements in hospitalized patients with COVID-19. Blood 2020;136(6):766–8. https://doi.org/10.1182/blood.2020006695.
[16] Angileri F, Légaré S, Marino Gammazza A, Conway De Macario E, Macario AJL, et al. Is molecular mimicry the culprit in the autoimmune haemolytic anaemia affecting patients with COVID-19? Br J Haematol 2020;190(2):e92–3. https://doi.org/10.1111/bjh.16883.
[17] Diao B, Wang C, Wang R, Feng Z, Tan Y, et al. Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Nat Commun 2021;12(1):2506. https://doi.org/10.1038/s41467-021-22781-1.

[18] Aiello S, Gastoldi S, Galbusera M, Ruggenenti P, Portalupi V, et al. C5a and C5aR1 are key drivers of microvascular platelet aggregation in clinical entities spanning from aHUS to COVID-19. Blood Adv 2022;6(3):866–81. https://doi.org/10.1182/bloodadvances.2021005246.

[19] Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. J Clin Invest 2020;130(11):6151–7. https://doi.org/10.1172/JCI141374.

[20] Frimat M, Tabarin F, Dimitrov JD, Poitou C, Halbwachs-Mecarelli L, et al. Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome. Blood 2013;122(2):282–92. https://doi.org/10.1182/blood-2013-03-489245.

[21] Jäger U, Barcellini W, Broome CM, Gertz MA, Hill A, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the first international consensus meeting. Blood Rev 2020;41:100648. https://doi.org/10.1016/j.blre.2019.100648.

[22] Fattizzo B, Zaninoni A, Nesa F, Sciumbata VM, Zanella A, et al. Lessons from very severe, refractory, and fatal primary autoimmune hemolytic anemias: severe, refractory and fatal autoimmune hemolytic anemias. Am J Hematol 2015;90(8):E149–51. https://doi.org/10.1002/ajh.24047.

[23] Risitano AM, Mastellos DC, Huber-Lang M, Yancopoulou D, Garlanda C, et al. Complement as a target in COVID-19? Nat Rev Immunol 2020;20(6):343–4. https://doi.org/10.1038/s41577-020-0320-7.

[24] Kononowicz JE, Farhan Ali M, Palko W, Pyper S, Agasthya N. Rapidly progressing autoimmune hemolytic anemia in a pediatric patient with COVID-19. Cureus 2023. https://doi.org/10.7759/cureus.45633.

[25] Hsieh T-C, Sostin O. Severe warm autoimmune hemolytic anemia in COVID-19 managed with least incompatible rbc product and glucocorticoids. Ann Hematol 2022;101(2):431–2. https://doi.org/10.1007/s00277-021-04457-4.

[26] Fattizzo B, Pasquale R, Bellani V, Barcellini W, Kulasekararaj AG. Complement mediated hemolytic anemias in the COVID-19 era: case series and review of the literature. Front Immunol 2021;12:791429. https://doi.org/10.3389/fimmu.2021.791429.