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The significance of antiglobulin (Coombs) test reactivity in patients with COVID-19

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ABSTRACT

Previous case reports have described patients with COVID-19-associated autoimmune hemolytic anemia (AIHA), and cold agglutinin disease (CAD) which is characterized by a positive direct antiglobulin (DAT) or "Coombs" test, yet the mechanism is not well understood. To investigate the significance of Coombs test reactivity among COVID-19 patients, we conducted a retrospective study on hospitalized COVID-19 patients treated at NMC Royal Hospital between 15 April and 30 May 2020. There were 27 (20%) patients in the Coombs-positive group and 108 (80%) in the Coombs-negative group. The cold agglutinin titer was examined in 22 patients due to symptoms suggestive of cold agglutinin disease, and all tested negative. We demonstrated a significant association with reactive Coombs test results in univariate analysis through clinical findings such as ICU admission rate, the severity of COVID-19, and several laboratory findings such as CRP, D-dimer, and hemoglobin levels lactate dehydrogenase, and RDW-CV. However, only hemoglobin levels and disease severity had a statistically significant association in multivariate analysis. A possible explanation of COVID-19-associated positive Coombs is cytokine storm-induced hyperinflammation, complement system activation, alterations of RBCs, binding of SARS-CoV-2 proteins to hemoglobin or its metabolites, and autoantibody production. Coombs-positive patients were tested for hemolysis using indirect bilirubin, consumed haptoglobin, and/or peripheral smear that ruled out any evidence of hemolysis. Understanding this etiology sheds new light on RBC involvement as a pathophysiological target for SARS-CoV-2 by interfering with their function; consequently, therapies capable of restoring RBC function, such as erythrocytapheresis, could be repurposed for the treatment of worsening severe and critical COVID-19.

1. Introduction

The coronavirus disease 2019 (COVID-19) outbreak first emerged in Wuhan city, China, at the end of 2019. Symptoms of COVID-19 range from being asymptomatic with no effect on different biological activities to mild symptoms, including fever, dry cough, diarrhea, and vomiting. The disease can also be severe or critical, leading to pneumonia, acute respiratory distress syndrome (ARDS), multiorgan failure, and death (Chen et al., 2020a).

COVID-19 is associated with different abnormal clinical laboratory findings, including thrombocytopenia, elevated D-dimer, lactate dehydrogenase (LDH), low hemoglobin (Hb), abnormal red blood cell distribution width (RDW-CV), bilirubin, and increased polymorphonuclear to lymphocyte ratio, especially with increasing severity of the disease (Chen et al., 2020c; Wang et al., 2020; Huang et al., 2020; Sheng et al., 2021).

Accumulating evidence also suggests an aggregated immune response and cytokine storm contribution to the severe form of COVID-19. It is associated with higher admission rates to the intensive care unit (ICU) and mortality (Mahmudpour et al., 2020). Chen et al. found that patients who died due to COVID-19 had higher levels of interleukins (ILs) and immune modulators, including IL-2, IL-6, IL-8, IL-10, and tumor necrosis factor-alpha (TNFα), than recovered patients (Chen et al., 2020b).

There are several mechanisms by which inflammation could interfere with erythropoiesis, either by alteration of iron metabolism caused

Abbreviations: AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; DAT, direct antiglobulin test; RDW-CV, red blood cell distribution width.

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by overproduction of IL-6 (Nemeth & Ganz, 2014) or by reduction of erythrocyte lifespan and inhibition of erythroid progenitor and precursor cells by IL-33 and interferon γ (IFN-γ) (Libregts et al., 2011; Swann et al., 2020).

On the other hand, a few case reports have described patients with COVID-19-associated autoimmune hemolytic anemia (AIHA) and cold agglutinin disease (CAD). However, the mechanism is not yet well understood (Jawed et al., 2020; Lopez et al., 2020; Zagorski et al., 2020). AIHA is characterized by autoimmune antibodies directed against antigens present on the patients' red blood cells (RBCs) with subsequent complement system activation. Autoantibodies can deposit at an optimal temperature of 37 °C, causing warm AIHA followed by sequestration and phagocytosis of warm antibody-coated RBCs (Jandl et al., 1957; Kurlander & Rosse, 1979; Jandl & Kaplan, 1960). Warm autoantibodies are mostly immunoglobulin G (IgG) and less frequently IgA either in combination with IgG or alone (Packman, 2008; Janvier et al., 2002).

Cold agglutinin disease (CAD) is associated with IgM autoantibodies produced by B cells that result in agglutination of RBCs by antigen–antibody reactions and complement system activation between 0 and 4 °C (Hill & Hill, 2018). The disease will be pathogenic, usually of the IgM class, if the thermal amplitude exceeds 28–30 °C (Harboe & Deverill, 1964; Berentsen et al., 2006). CAD is different from cold agglutinin syndrome (CAS). Primary CAD is usually not associated with the presence of underlying diseases such as lymphomas or other malignancies. CAD is now considered a well-defined clinicopathological disorder; hence, it is a disease, not a syndrome (Berentsen, 2016). While CAS is considered uncommon compared to primary CAD, it usually presents as a complication to other diseases, such as diffuse large B-cell lymphoma, Hodgkin's lymphoma, or Epstein–Barr virus infection (Berentsen & Sundic, 2015).

The direct antiglobulin test (DAT) or direct Coombs test is widely used to diagnose AIHA and CAS. It depends on the detection of an antibody isotype, usually immunoglobulin G (IgG) ± complement (C3d), that coating cell membrane of RBCs at 37 °C in cases of warm AIHA. CAIHA results in a DAT positive for C3d and negative for IgG (Zantek et al., 2012). However, DAT negativity could be obtained in 3 to 10% of warm-AIHA patients (Packman, 2008; Sachs et al., 2006). DAT remains the most accurate diagnostic tool for AIHA (Lai et al., 2013).

A recent study showed that anemia affected 61% of COVID-19 patients primarily due to inflammation (Bergamaschi et al., 2021), highlighting the importance of understanding the underlying causes and consequences as well as choosing optimal medical interventions against anemia caused by COVID-19.

This study aimed to investigate the significance of Coombs test reactivity in relation to the pathophysiology, clinical presentation, degree of disease severity, and mortality due to COVID-19.

2. Method

2.1. Study design and study population

This was a noninterventional retrospective study of medical records from patients with COVID-19 treated in NMC Royal Hospital, Khalifa City, Abu Dhabi, UAE, between April 15th and May 30th, 2020. Included patients in the study were hospitalized adult COVID-19 patients confirmed by real-time reverse transcription-polymerase chain reaction (RT–PCR) assay developed from the publicly released virus sequence by nasopharyngeal swab under aseptic operation.

Inclusion criteria included hospitalized adult patients with COVID-19 (≥18 years) with different disease severity grades admitted in the tentative period, with no exclusions.

Patient identifiers were removed while processing the data, with complete patient privacy protection. This study was conducted according to the Declaration of Helsinki. The study was reviewed and approved by the Central Scientific Committee NMC (NMCHC/CSC/2020/0035), NMC Regional Research Ethics Committee (NMC/PREC/AUH/2021/0002), and COVID-19 Research Ethics Committee, Department of Health, Abu Dhabi, UAE (Ref: DOH/CVDC/2020/2467).

2.2. SARS-CoV-2 nasopharyngeal PCR test

RNA was extracted from the nasopharyngeal swabs using the Xybio extraction kit, Korea. RT–PCR was performed on the Bio-Rad Cycler PCR, USA, using Solgent's 2019-nCoV Real-Time Reverse Transcription PCR Kit, following the manufacturer's instructions. Viral detection was performed using a CFX96 plate reader from Bio-Rad in the United States. A cycle threshold value (Ct value) <40 was defined as a negative result, whereas a Ct value greater than 40 was considered a positive test based on the cut of CT values of 40 for the N gene and the Orf gene.

2.3. Direct Coombs test

Because some patients had high lactate dehydrogenase levels, the anti-globulin test (Coombs test) was conducted as part of the usual workup for the general assessment of COVID-19 patients with varying severity grades.

The direct Coombs test was performed using column agglutination technology of the ORTHO BioVue system, which consists of glass beads and a reagent enclosed in a column that traps when the cassette is centrifuged agglutinated red blood cells while allowing nonagglutinated red blood cells to travel to the bottom of the column. Red cells are isolated from serum proteins prior to exposure to the polyspecific anti-human globin reagent that can detect both IgG and C3. The reagent's density allows red blood cells to flow through the column while less dense neutralizing serum proteins remain above the glass bead/reagent interface. The Coomb's test conducted by the laboratory is validated as a "test methodology" that is further accredited for the ISO 15189:2012 standards. Moreover, the validation of the test is done for the test's "methodology," which assures the particular methodology provides confidence in releasing results.

2.4. Cold agglutinin titer test

The agglutinin titer test was performed in the National Reference Laboratory in UAE, order Code number (006353). Blood samples were obtained from some of the patients and incubated at 37 °C. Blood clotting was allowed at room temperature or 37 °C. Then, the serum was isolated immediately and stored in the refrigerator to perform the agglutination test. The cutoff value for the cold agglutinin test was 1:32. Cold agglutinin titers exceeding 1:64 dilutions were used as an indicator of CAD. This test was developed by LabCorp Burlington, 1447 York Cort, Burlington, NC 27215-3361.

2.5. Data collection

Demographic and clinical characteristics, laboratory findings, radiography, treatment, and outcomes were retrieved from the electronic medical records.

Patients were diagnosed with COVID-19 using RT–PCR assay with nasopharyngeal swabs. The Coombs test was performed as part of the routine workup for the general assessment of COVID-19 patients with varied severity categories after high lactate dehydrogenase levels were observed in some patients with no apparent underlying cause. Baseline laboratory tests were performed at the time of/and during admission, including complete blood count (CBC), C-reactive protein (CRP), D-dimer, LDH, liver function tests, kidney function tests, lymphocytic count, coagulation profile (prothrombin time, aPTT, fibrinogen), IL-6 and serum ferritin. Hemolysis investigations were performed on Coombs-positive patients, including indirect bilirubin, haptoglobin, and/or peripheral smears.

The serum IL-6 test was performed in the National Reference

Laboratory in UAE under the order code 140916. IL-6 was detected by an enzyme-linked immunosorbent assay (ELISA) with a reference range of 0.0–15.5 pg/mL. The test was developed by LabCorp Burlington, 1447 York Cort, Burlington, NC 27215-3361.

All patients had X-ray chest and/or chest CT scans at the time of admission, and some of them had follow-up X-ray chest and/or chest CT scans within different time intervals according to clinical assessment.

The severity of COVID-19 was determined according to WHO/UAE early guidelines for the COVID-19 severity scale. Mild cases are symptomatic COVID-19 patients without any symptoms of hypoxia or pneumonia. Moderate COVID-19 patients are those with pneumonia symptoms but SpO2 $\geq$ 93% on room air. A severe case of COVID-19 was defined as the presence of moderate disease symptoms with one of the following symptoms of pneumonia: severe respiratory distress, respiratory rate greater than 30 breaths/min, or SpO2 $<$ 93% on room air. Critical cases are those developing acute respiratory distress syndrome (ARDS), which is defined by the onset of pneumonia within 1 week of a known clinical insult or new or worsening respiratory symptoms in addition to multiorgan failure, sepsis, and shock (MOHAP ICU Team, 2020).

2.6. Statistical analysis

Continuous variables were described as the means with standard deviation, median, minimum, maximum, and interquartile range (IQR). Categorical variables were described as frequencies (n) and percentages. The Chichi-square test was used to study the correlation between the Coombs test and the degree of disease severity and mortality. An independent *t* test (Mann–Whitney test) was used to study the difference between Coombs negative and positive groups regarding their clinical laboratory findings. The chi-square test was used to study the association between categorical variables and the Coombs test. The Mann–Whitney test, a nonparametric test, was used for comparative analysis between positive and negative Coombs test COVID-19 patients regarding continuous variables that violated normal assumptions. The Anderson Darling test of normality was used to assess the normality of variables. The logistic regression model was used to determine the independent association of the Coombs test with COVID-19 severity, mortality, and clinical-laboratory variables that were significant in univariate analysis. All analyses were performed using IBM SPSS (version 26.0; IBM Corp., Armonk, NY, USA). A two-sided p value $<$ 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics of the study population

A total of 135 patients hospitalized with COVID-19 were included in the study, of whom 113 (83.7%) were males, and 107 (79.3%) were Asian. The mean age was 41.78 $\pm$ 10.5 years old. We stratified patients according to Coombs test results. There were 27 (20%) patients in the Coombs-positive group and 108 (80%) in the Coombs-negative group. The majority were males, accounting for 21 (77.8%) patients, while only six (22.2%) were females. Comorbidities were present in 60 (29.6%) patients, of which diabetes mellitus (DM) accounted for 27 (20%), and hypertension accounted for 25 (18%) patients. Pneumonia was present in 23 (85.2%). Severe cases of COVID-19 accounted for 16 (59.3%) patients, and nine (33.3%) were admitted to the ICU and needed invasive mechanical ventilation. Twenty-two patients who presented with symptoms suggesting CAD were negative for cold agglutinin titers.

In the positive Coombs test group, death accounted for six (22.2%) patients (Table 1). According to the chi-square test, there was an association between positive Coombs test results and the rate of ICU admission (P = 0.014), the need for invasive mechanical ventilation (P = 0.005), the severity of the disease (P = 0.004), WHO ordinary scale (P = 0.009), and mortality (P = 0.005). Other patient characteristics were not significantly associated with the Coombs test results (Table 1).

Table 1
Characteristics of patients with COVID-19 stratified by Coombs Test. (Data are presented as n, and %.).

Patient Characteristics		COOMB TEST				P value
		Negative		Positive		
Gender	Male	92	85.2%	21	77.8%	0.385
	Female	16	14.8%	6	22.2%	
Race	Asian	85	78.7%	22	81.5%	0.597
	White	19	17.6%	5	18.5%	
	Black	4	3.7%	0	0.0%	
HTN	Yes	17	15.7%	8	29.6%	0.097
	No	91	84.3%	19	70.4%	
DM	Yes	22	20.4%	5	18.5%	0.830
	No	86	79.6%	22	81.5%	
CVS	Yes	4	3.7%	4	14.8%	0.051
	No	104	96.3%	23	85.2%	
Radiology	Normal	34	31.5%	4	14.8%	0.085
	Pneumonia	74	68.5%	23	85.2%	
Blood Group	A	28	31.5%	6	35.3%	0.106
	AB	3	3.4%	3	17.6%	
	B	24	27.0%	4	23.5%	
	O	34	38.2%	4	23.5%	
RH	Positive	69	90.8%	16	94.1%	1.000
	Negative	7	9.2%	1	5.9%	
ICU Admission	Yes	12	11.1%	9	33.3%	**0.014**
	No	96	88.9%	18	66.7%	
HF-NIV	Yes	16	14.8%	4	14.8%	1.000
	No	92	85.2%	23	85.2%	
Invasive Mechanical Ventilation	Yes	4	3.7%	6	22.2%	**0.005**
	No	104	96.3%	21	77.8%	
Ventilated	Yes	20	18.5%	10	37.0%	**0.038**
	No	88	81.5%	17	63.0%	
Multiple Organ Failure	Yes	2	1.9%	5	18.5%	**0.004**
	No	106	98.1%	22	81.5%	
Died	Yes	4	3.7%	6	22.2%	**0.005**
	No	104	96.3%	21	77.8%	
Severity	Severe	32	29.6%	16	59.3%	**0.004**
	Non-Severe	76	70.4%	11	40.7%	
Severity	Non-Severe	76	70.4%	11	40.7%	**0.000**
	Severe	10	9.3%	5	18.5%	
	Early Critical	21	19.4%	5	18.5%	
	Late Critical	1	0.9%	6	22.2%	
WHO Ordinary Scale	2	35	32.4%	6	22.2%	**0.009**
	3	43	39.8%	6	22.2%	
	4	11	10.2%	4	14.8%	
	5	19	17.6%	9	33.3%	
	6	0	0.0%	2	7.4%	
Evidence of Hemolysis	Yes	0	0%	0	0%	–
	No	108	100%	27	100%	–

HTN: Hypertension; DM: Diabetes Mellitus; CVS; Cardiovascular Disease; RH: Rhesus Factor; ICU: Intensive Care Unit; HF-NIV: High Flow Oxygen and Non-Invasive Ventilation

3.2. The association between clinical laboratory findings and Coombs test results

We found a statistically significant association between the results of the Coombs test and hemoglobin levels (median = 12.8 IQR = [11.1–14.5] vs. 14.3 [13.3–15.3], P $<$ 0.000) in the positive Coombs test group and negative Coombs test group, respectively. Other laboratory findings that showed statistically significant association with Coombs test results while comparing positive Coombs test and negative Coombs test were CRP levels (89 [7 – 164] vs. 18.5 [4–84.25], P $<$ 0.006), D-Dimer (1.11 [0.27–7.91] vs. 0.4 [0.23–0.975], P $<$ 0.008), and Fibrinogen levels (698 [512 – 840] vs. 512.5 [330.25–663.75], P $<$ 0.002) Lactate Dehydrogenase (258.5 [194 – 381] vs. 512.5 [330.25–663.75], P $<$ 0.001) (Table 2).

On the other hand, there was no association between Coombs test results and IL-6 (P = 0.82), time to viral clearance (P = 0.207),

Table 2
Univariate comparative analysis between positive and negative Coombs's tests regarding baseline laboratory parameters (data are represented as the median & IQR).

Parameter	COOMB TEST		P value
	Negative	**Positive**	
Age	41.5 (33–48)	43 (38–52)	0.154
BMI	27.485 (24.6925–29.985)	27.505 (23.61–30.18)	0.760
WBC	6.635 (5.2–8.14)	5.9 (4.5–7.2)	0.120
Hemoglobin	14.3 (13.3–15.3)	12.8 (11.1–14.5)	**0.000**
Platelets	279 (216.75–377.75)	281 (224–427)	0.518
CRP	18.5 (4–84.25)	89 (7–164)	**0.006**
D-Dimer	0.4 (0.23–0.975)	1.11 (0.27–7.91)	**0.008**
IL-6	29.65 (12.8–91.65)	98.5 (35.5–1432.1)	0.082
LDH	258.5 (194–381)	459 (251–572)	**0.001**
ALT	41.5 (26.75–64.25)	42 (29–70)	0.759
AST	37 (25–52.75)	47 (32.75–61.25)	**0.017**
Creatinine	0.875 (0.7425–1.0175)	0.82 (0.61–1.11)	0.464
Neutrophil Count	60.4 (51.15–72.925)	76.6 (59.5–84.4)	**0.008**
Lymphocyte Count	28.3 (17.7–37.65)	16.6 (11.2–30.5)	**0.007**
NLR	2.14 (1.3475–4.31)	4.6 (1.7–7.3)	**0.007**
RDW-CV	12.8 (12.3–13.2)	13.7 (13–15.6)	**0.000**
Fibrinogen	512.5 (330.25–663.75)	698 (512–840)	**0.002**
Ferritin	358.8 (155.3–807.6)	919 (64–1666)	0.173
PT	14 (13–14.8)	14 (13–15)	0.225
INR	1 (0.95–1.07)	1.05 (0.92–1.11)	0.242
TROPI	0.002 (0.002–0.01)	0.01 (0.002–0.01)	0.491
PCT	0.05 (0.02–0.08)	0.07 (0.04–0.0925)	0.195
GLU	5.89 (5–8.36)	5.5 (5.1–6.95)	0.709
Time to Viral Clearance	17 (11–26.75)	20 (13–30)	0.207

BMI: Body Mass Index; WBC: White Blood Cells; CRP: C-reactive Protein; IL-6: Interlukin-6; LDH: Lactate Dehydrogenase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; NLR: Neutrophil-to-Lymphocyte Ratio; RD-WCV: Red Blood Cell Distribution Width; PT: Prothrombin Time; INR: International Normalized Ratio; TROP I: Troponin I ; PCT: Procalcitonin; GLU: Glucose

creatinine levels (P = 0.464), or PT (P = 0.225) (Table 2).

3.3. Logistic regression analysis

The univariate logistic regression models showed that the unadjusted odds of positive Coombs test results increased significantly among ICU-admitted COVID-19 patients by approximately 4-fold compared to patients who were not admitted to the ICU (OR = 4.00, 95% CI: [1.47–10.88], p = 0.007). The odds of positivity increased significantly among patients undergoing invasive mechanical ventilation by approximately 7.4-fold compared to those who did not (OR = 7.43, 95% CI: [1.93–28.63], p = 0.004). Additionally, the unadjusted odds of positivity increased significantly among patients with multiple organ failure by approximately 12-fold (OR = 12.05, 95% CI: [2.19–66.13], p = 0.004).

Regarding mortality, the unadjusted odds of positivity showed a statistically significant increase among dead cases by approximately 7.4-fold compared to improved ones (OR = 7.43, 95% CI: [1.93–28.63], p = 0.004). The odds of positivity increased significantly by approximately 3.5-fold among severe cases (OR = 3.45, 95% CI: [1.44–8.26], p = 0.005).

Regarding laboratory parameters, the unadjusted odds of positivity decreased significantly by approximately 32% for each one-unit increase in hemoglobin level (OR = 0.68, 95% CI: [0.52–0.88], p = 0.003). The odds of positivity increased significantly by approximately 1%, 4%, 9%, and 50% for each one-unit increase in CRP level, neutrophil count, NLR, and RDW-CV, respectively (OR = 1.01, 95% CI: [1.00–1.01], p = 0.014), (OR = 1.04, 95% CI: [1.01–1.07], p = 0.008), (OR = 1.09, 95% CI: [1.01–1.18], p = 0.025) and (OR = 1.50, 95% CI: [1.15–1.94], p = 0.003), respectively. On the other hand, the odds of positivity decreased significantly by approximately 5% for each one-unit increase in lymphocyte count (OR = 0.95, 95% CI: [0.92–0.99], p = 0.011).

The multivariate logistic regression model showed that the adjusted odds of Coombs test positivity increased significantly among severe COVID-19 cases by approximately 3-fold compared to nonsevere ones (OR = 3.03, 95% CI: [1.23–7.45], p = 0.016), while the adjusted odds of positivity decreased significantly by approximately 32% for each one-unit increase in hemoglobin level (OR = 0.68, 95% CI: [0.52–0.90], p = 0.007). (Table 3).

4. Discussion

We identified a correlation between a reactive Coombs test at presentation and disease progression in COVID-19 patients. Lower hemoglobin levels, a severe disease course, higher levels of inflammatory markers, and LDH were all linked to a reactive Coombs test. In our study, the prevalence of Coombs positivity was 20% in COVID-19 patients in our health facility, which is higher than that in hospitalized non-COVID-19 patients (7:8%) or healthy blood donors (1 in 1,000–1:14,000) (Zantek et al., 2012).

Our findings are consistent with Berzuini et al., who reported Coombs positivity in 46% of COVID-19 patients, 88% positivity for IgG only, and 8% positivity for IgG and C3d (Berzuini et al., 2020).

The prevalence of positive Coombs tests in our study was slightly lower than in the Berzuini et al. study, which could be attributed to the less severe patients' conditions in our study or the fact that not all Coombs positive tests in the Berzuini et al. study were related to SARS-CoV-2 infection, where some positive Coombs tests could be attributed to medications provided. Although cold agglutinin disease could be attributed to the weather differences between the UAE and Europe, positive IgG and C3d patterns in the Berzuini study suggest that cold agglutinin disease is an unlikely explanation.

Platton et al. compared Coombs positivity between the SARS-CoV-2-positive group and SARS-CoV-2-negative group and showed a positive Coombs test in 80% of COVID-19 patients compared with 35% in the control group (P = 0.004) with no evidence of AIHA. These data indicate that positive Coombs results are related to SARS-CoV-2 infection, although the mechanism of this association is not well understood (Platton et al., 2021).

Even though the underlying mechanisms underlying positive DAT among COVID-19 patients are currently unknown, several hypotheses

Table 3
Univariate & multivariate logistic regression models (Data are represented as OR, (95% CI)).

Patient Characteristics	Univariate ODDS Ratio (95% CI)	p value	Multivariate ODDS Ratio (95% CI)	p value
ICU- Admission (Yes)	4.00 (1.47–10.88)	**0.007**		
Invasive Mechanical Ventilation (Yes)	7.43 (1.93–28.63)	**0.004**		
Multiple organ failure (Yes)	12.05 (2.19–66.13)	**0.004**		
Died (Yes)	7.43 (1.93–28.63)	**0.004**		
Disease Severity (Severe)	3.45 (1.44–8.26)	**0.005**	3.03 (1.23–7.45)	**0.016**
Severity Index (Non-Severe)	1.00 (0.00–0.00)	**0.004**		
Hemoglobin	0.68 (0.52–0.88)	**0.003**	0.68 (0.52–0.90)	**0.007**
CRP	1.01 (1.00–1.01)	**0.014**		
Neutrophil- Count	1.04 (1.01–1.07)	**0.008**		
Lymphocyte-Count	0.95 (0.92–0.99)	**0.011**		
NLR	1.09 (1.01–1.18)	**0.025**		
RDW_CV	1.50 (1.15–1.94)	**0.003**		
Fibrinogen	1.00 (1.00–1.01)	**0.004**		

CRP: C-reactive Protein; NLR: Neutrophil-to-Lymphocyte Ratio; RD-WCV: Red Blood Cell Distribution Width.

have been proposed, such as the molecular mimicry proposed by Angilleri et al. suggesting autoimmunity against erythrocytes based on molecular mimicry of ankyrin 1 (ANK-1) protein, which is present in the erythrocyte membrane, to an antigenic epitope of spike (S) protein in SARS-CoV-2 (Angileri et al., 2020).

Here, a positive Coombs test was observed to be associated with severe disease outcomes and elevated inflammatory markers related to the cytokine storm reflecting that hyperinflammation and alteration of RBC membranes with exposure to cryptic antigens could be another possible underlying mechanism for a positive Coombs test (Hendrickson and Tormey, 2020; Berentsen, 2020). Autoantibodies against type I interferon were also observed among COVID-19 patients with life-threatening COVID-19 pneumonia (Bastard et al., 2020, p. 1).

On the other hand, Berzuini et al. reported that eluates from DAT-positive COVID-19 patients (IgG separated from patients' RBCs) reacted with RBCs from DAT-negative COVID-19 patients and not with standard reagent RBCs, suggesting that SARS-CoV-2 infection could lead to alterations of the membrane of RBCs (Berzuini et al., 2020). They also reported two COVID-19 patients positive for C3d only (Berzuini et al., 2020). This could be due to the activation of mannose-binding lectin-associated serine protease-2 by the nucleocapsid (N) protein of SARS-CoV-2 and, subsequently, C4 cleavage and activation of the complex pathway followed by accumulation of C3d (Hendrickson and Tormey, 2020).

We conducted the Coombs test at the time of admission to the hospital before initiation of any treatments against COVID-19 before hospitalization, confirming that positive Coombs test results in our study were likely to be related to SARS-CoV-2 infection and not induced by the drugs. In contrast to the study of Berzuini et al., where the Coombs test was performed after hospitalization and treatment initiation with different drug categories, including antivirals and hydroxychloroquine.

We also observed a significant association between the severity of COVID-19 and the positivity of the Coombs test ($P = 0.0016$), in addition to higher rates of ICU admission in the Coombs-positive group (33.3% in the Coombs-positive group vs. 11.1% in the Coombs-negative group, $P = 0.014$). Algassim et al. also reported higher rates of ICU admission and mortality (32%) among DAT-positive COVID-19 patients (Algassim et al., 2021).

In our study, several laboratory findings showed a significant association with Coombs test results in univariate analysis, such as CRP, D-dimer, hemoglobin levels, LDH, and RDW-CV, and clinical findings, such as the rate of ICU admission and severity of COVID-19. However, only hemoglobin levels and disease severity had a statistically significant association in multivariate analysis. Berzuini et al. reported even lower hemoglobin levels and a significant association between a positive Coombs test and hemoglobin levels ($P < 0.01$). Moreover, Berzuini et al. also indicated that 51.9% of Coombs-positive patients required at least one blood transfusion ($P = 0.009$) (Berzuini et al., 2020). In contrast, Platton et al. found no difference between hemoglobin levels between COVID-19 patients and non-COVID-19 patients (Platton et al., 2021).

A systematic review and meta-analysis conducted by Ghahramani et al. reported lower hemoglobin levels in severe compared to nonsevere COVID-19 patients (Ghahramani et al., 2020). Algassim et al. also reported a higher prevalence of decreased hemoglobin levels among COVID-19 patients admitted to the ICU than among those admitted to the general ward (37% vs. 16%). The decline in hemoglobin levels in severe COVID-19 patients could be attributed to inflammation caused by SARS-CoV-2 infection. Several mechanisms could account for this, including altered iron metabolism induced by immune modulators such as IL-1, IL-6, and activin B, followed by hepcidin production and decreased iron transfer for erythropoiesis. Another mechanism includes inhibiting erythropoietin (EPO) formation (Weiss et al., 2019).

Cavezzi et al. suggested that various mechanisms, including alteration of hemoglobin, could provoke hypoxia associated with COVID-19 through either heme metabolism inhibition or hemoglobin denaturation. Another possible mechanism is hepcidin mimicry by the spike protein of SARS-CoV-2 and blockage of ferroportin (the main iron exporter protein) (Cavezzi et al., 2020). RBC membrane alterations, complement effects, or medication effects are additional plausible explanations for the reactive Coombs test and its specific eluate reactivity reported in patients with COVID-19. (Hendrickson and Tormey, 2020).

Complement system dysregulation is involved in the pathophysiology of several thrombotic microangiopathy (TMA) disorders, including atypical hemolytic uremic syndrome (aHUS), thrombotic thrombocytopenic purpura (TTP), paroxysmal nocturnal hemoglobinuria (PNH), warm AIHA, and CAS (Gavriilaki et al., 2019).

PNH is caused by the loss of glycosylphosphatidylinositol (GPI)-anchored complement regulatory proteins, leading to uncontrolled complement system activation (Baines & Brodsky, 2017). aHUS usually results from a genetic abnormality in complement and complement regulatory proteins or the development of autoantibodies against complement factors leading to activation of the alternative complement pathway (Noris & Remuzzi, 2010). The clinical picture of TTP and Ahus overlap; however, the differential diagnosis between them is confirmed by determining a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13 protein (ADAMTS13) activity (Gavriilaki et al., 2019).

However, autoimmune hemolytic anemia is associated with the activation of the classical complement pathway. IgM is considered a potent complement activator, in contrast to IgG. Additionally, IgG3 is considered a more efficient activator of the complement system than IgG1 and IgG2. IgA does not activate the complement system (Berentsen & Sundic, 2015).

AIHA was reported among COVID-19 patients (Capes et al., 2020; Lazarian et al., 2020; Lopez et al., 2020). Algassim et al. found a positive DAT test and spherocytosis among 14% of anemic ICU-admitted COVID-19 patients compared to 9% of anemic general ward patients. Haptoglobin levels were not measured, but the diagnosis of AIHA was confirmed by the presence of spherocytes in blood films (Algassim et al., 2021).

This observation was not similar to ours. In our study, hemolysis was ruled out in Coombs-positive patients by examining bilirubin, haptoglobin, and/or peripheral smear. Haptoglobin was also investigated for possible consumption. It was unexpected that haptoglobin levels were higher than normal, which is presumably due to the ongoing acute-phase response to SARS-CoV-2.

In our study, 22 patients presented with CAD symptoms, but all of them were negative for cold agglutinin titer.

These observations indicate that a positive Coombs test among COVID-19 patients is not necessarily suggestive of AIHA or CAD. Nevertheless, it could indicate an altered RBC membrane and/or hemoglobinopathy leading to abnormal hemoglobin function. Spitalnik and his colleagues conducted a recent multiomics study to explore the effect of SARS-CoV-2 infection on red blood cells. They reported increased glycolysis, increased oxygenation and fragmentation of essential surface proteins, and altered lipid metabolism of RBC membranes. These alterations lead to RBC deformities that could contribute to the thromboembolism and coagulation caused by COVID-19. The alteration of the N-terminal cytosolic domain of band 3 (AE1) is responsible for deoxyhemoglobin stabilization and oxygen loading. These alterations, in turn, could impair RBCs' capabilities of oxygen transfer to the cells, leading to tissue hypoxia and organ damage observed among COVID-19 patients. They can also trigger the immune system to produce antibodies directed against altered RBCs (Thomas et al., 2020). Altered morphology and structure of RBCs could also contribute to the pathology of hemoglobinopathy (Kuypers, 2007).

Renoux et al. observed abnormal RBC rheological characteristics in COVID-19 patients; RBCs aggregated at low shear rates and stasis. Enhanced RBC aggregation may lead to blood clot formation, resistance, and an increased risk for thromboembolic events in patients with COVID-19. Red blood cell deformity (RBCD) was decreased in COVID-19 patients. This may be due to surface protein fragmentation and lipid

composition alterations. However, no difference in blood viscosity was observed among the different groups included in the study(Renoux et al., 2021).

Based on this hypothesis, we propose that treatment with erythrocytapheresis could effectively restore RBCs' oxygen-carrying capacity, maintain the normal function of RBCs in general, and improve oxygenation in severe and critical COVID-19. Positive outcomes of RBC transfusion were observed in an old COVID-19 patient with several comorbidities. The patient was intubated and then treated with packed RBC transfusion. On the second day, the patient was extubated, and his oxygen state was improved (Ejigu et al., n.d.).

5. Conclusion

In summary, hospitalized COVID-19 patients had a higher prevalence of a positive Coombs test, which several theories could explain, including cytokine storm-induced hyperinflammation, complement system activation, alterations of RBCs, binding of SARS-CoV-2 proteins to hemoglobin or its metabolites, and autoantibody production.

These findings extend our understanding of the pathophysiology of COVID-19 and open new insights for potential strategies for new interventions for critical COVID-19 patients. Future studies and controlled clinical trials are awaited to investigate the pathogenesis of COVID-19-associated Coombs test positivity, setting the cutoff values of hemoglobin required for packed RBC transfusions in patients with severe COVID-19, especially in cases with clinical findings suggesting the presence of progressive tissue hypoxia. Moreover, this could recommend the utility of some other potentially valuable strategies, such as erythrocytapheresis, in managing severe and critical COVID-19.

6. Limitations of the study

We would like to overcome some limitations to our study in future well-designed research. The study was retrospective and observational and had a relatively small sample size, predominantly male. Additionally, only polyspecific DAT was performed; monospecific DAT and detailed analysis of immunoglobulin classes and complement on erythrocytes were not performed.

7. Institutional review board statement

The study was reviewed and approved by the Central Scientific Committee NMC (NMCHC/CSC/2020/0035), NMC Regional Research Ethics Committee (NMC/PREC/AUH/2021/0002), and COVID-19 Research Ethics Committee, Department of Health, Abu Dhabi, UAE (Ref: DOH/CVDC/2020/2467).

8. Informed consent statement

This was a retrospective study; all patient identifiers were removed during the data collection process, with complete protection of patients' privacy. This study was conducted according to the Declaration of Helsinki and approved by IRB(s); the Central Scientific Committee NMC (NMCHC/CSC/2020/0035), NMC Regional Research Ethics Committee (NMC/PREC/AUH/2021/0002), COVID-19 Research Ethics Committee, Department of Health, Abu Dhabi, UAE (Ref: DOH/CVDC/2020/2467).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Algassim, A.A., Elghazaly, A.A., Alnahdi, A.S., Mohammed-Rahim, O.M., Alanazi, A.G., Aldhuwayhi, N.A., Alanazi, M.M., Almutairi, M.F., Aldeailej, I.M., Kamli, N.A., Aljurf, M.D., 2021. Prognostic significance of hemoglobin level and autoimmune hemolytic anemia in SARS-CoV-2 infection. Ann. Hematol. 100 (1), 37–43. https://doi.org/10.1007/s00277-020-04256-3.

Angileri, F., Légaré, S., Marino Gammazza, A., Conway de Macario, E., Macario, A. J. L., & Cappello, F. (2020). Is molecular mimicry the culprit in the autoimmune haemolytic anaemia affecting patients with COVID-19? In *British Journal of Haematology* (Vol. 190, Issue 2, pp. e92–e93). Blackwell Publishing Ltd. 10.1111/bjh.16883.

Baines, A.C., Brodsky, R.A., 2017. Complementopathies. Blood Rev. 31 (4), 213–223. https://doi.org/10.1016/j.blre.2017.02.003.

Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.-H., Zhang, Y.u., Dorgham, K., Philippot, Q., Rosain, J., Béziat, V., Manry, J., Shaw, E., Haljasmägi, L., Peterson, P., Lorenzo, L., Bizien, L., Trouillet-Assant, S., Dobbs, K., de Jesus, A.A., et al., 2020. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science (New York, N.Y.) 370 (6515). https://doi.org/10.1126/science.abd4585.

Berentsen, S., 2016. Cold agglutinin disease. Hematology 2016 (1), 226–231. https://doi.org/10.1182/asheducation-2016.1.226.

Berentsen, S., 2020. New insights in the pathogenesis and therapy of cold agglutinin-mediated autoimmune hemolytic anemia. Front. Immunol. 11, 590. https://doi.org/10.3389/fimmu.2020.00590.

Berentsen, S., Sundic, T., 2015. Red blood cell destruction in autoimmune hemolytic anemia: role of complement and potential new targets for therapy. Biomed Res. Int. 2015, 1–11.

Berentsen, S., Ulvestad, E., Langholm, R., Beiske, K., Hjorth-Hansen, H., Ghanima, W., Sørbø, J.H., Tjønnfjord, G.E., 2006. Primary chronic cold agglutinin disease: A population based clinical study of 86 patients. Haematologica 91 (4), 460–466.

Bergamaschi, G., Borrelli de Andreis, F., Aronico, N., Lenti, M.V., Barteselli, C., Merli, S., Pellegrino, I., Coppola, L., Cremonte, E.M., Croce, G., Mordà, F., Lapia, F., Ferrari, S., Ballesio, A., Parodi, A., Calabretta, F., Ferrari, M.G., Fumoso, F., Gentile, A., Melazzini, F., Di Sabatino, A., Bertolino, G., Codega, S., Costanzo, F., Cresci, R., Derosa, G., Stefano, M.D., Falaschi, F., Iadarola, C., Lovati, E., Lucotti, P.C., Martignoni, A., Mengoli, C., Miceli, E., Mugellini, A., Muggia, C., Noris, P., Pagani, E., Palumbo, I., Pecci, A., Perrone, T., Pieresca, C., Preti, P.S., Russo, M.C., Sgarlata, C., Siciliani, L., Staniscia, A., Vjera, F.T., Achilli, G., Agostinelli, A., Antoci, V., Banfi, F., Benedetti, I., Brattoli, M., Cambiè, G., Canta, R., Cococcia, S., Conca, F., Delliponti, M., Rio, V.D., Terlizzi, F.D., Fiengo, A., Forni, T., Freddi, G., Frigerio, C., Fusco, A., Gabba, M., Garolfi, M., Gori, G., Grandi, G., Grimaldi, P., Lampugnani, A., Lepore, F., Lettieri, G., Mambella, J., Mercanti, C., Nardone, A., Pace, L., Padovini, L., Pitotti, L., Reduzzi, M., Rigano, G., Rotola, G., Sabatini, U., Salvi, L., Santacroce, G., Savioli, J., Soriano, S., Spataro, C., Stefani, D., 2021. Anemia in patients with Covid-19: Pathogenesis and clinical significance. Clin. Exp. Med. 21 (2), 239–246.

Berzuini, A., Bianco, C., Paccapelo, C., Bertolini, F., Gregato, G., Cattaneo, A., Erba, E., Bandera, A., Gori, A., Lamorte, G., Manunta, M., Porretti, L., Revelli, N., Truglio, F., Grasselli, G., Zanella, A., Villa, S., Valent, L., & Daniele, A. (2020). Red cell–bound antibodies and transfusion requirements in hospitalized patients with COVID-19. *Blood*, *136*(6), 766–768. I 10.1182/blood.2020006695.

Capes, A., Bailly, S., Hantson, P., Gerard, L., Laterre, P.-F., 2020. COVID-19 infection associated with autoimmune hemolytic anemia. Ann. Hematol. 99 (7), 1679–1680.

Cavezzi, A., Troiani, E., Corrao, S., 2020. COVID-19: Hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. *Clin. Practice* 10 (2), 1271. https://doi.org/10.4081/cp.2020.1271.

Chen, Y., Liu, Q., Guo, D., 2020a. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J. Med. Virol. 92 (4), 418–423. https://doi.org/10.1002/jmv.25681.

Chen, T., Wu, D., Chen, H., Yan, W., Yang, D., Chen, G., Ma, K., Xu, D., Yu, H., Wang, H., Wang, T., Guo, W., Chen, J., Ding, C., Zhang, X., Huang, J., Han, M., Li, S., Luo, X., Ning, Q., 2020b. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. BMJ 368, m1091. https://doi.org/10.1136/bmj.m1091.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., Zhang, L.i., 2020c. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. The Lancet 395 (10223), 507–513.

Ejigu, T., Patel, N., Sharma, A., Vanjarapu, J. M. R., & Nookala, V. (n.d.). Packed red blood cell transfusion as a potential treatment option in COVID-19 patients with hypoxemic respiratory failure: a case report. *Cureus*, *12*(6), e8398. 10.7759/cureus.8398.

Gavriilaki, E., Anagnostopoulos, A., Mastellos, D.C., 2019. Complement in thrombotic microangiopathies: unraveling Ariadne's thread into the labyrinth of complement therapeutics. Front. Immunol. https://doi.org/10.3389/fimmu.2019.00337.

Ghahramani, S., Tabrizi, R., Lankarani, K.B., Kashani, S.M.A., Rezaei, S., Zeidi, N., Akbari, M., Heydari, S.T., Akbari, H., Nowrouzi-Sohrabi, P., Ahmadizar, F., 2020.

Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: A systematic review and meta-analysis. Eur. J. Med. Res. 25, 30. https://doi.org/10.1186/s40001-020-00432-3.

Harboe, M., Deverill, J., 1964. Immunochemical properties of cold haemagglutinins. Scandin. J. Haematol. 1 (3), 223–237. https://doi.org/10.1111/j.1600-0609.1964.tb00019.x.

Hendrickson, J.E., Tormey, C.A., 2020. COVID-19 and the coombs test. Blood 136 (6), 655–656. https://doi.org/10.1182/blood.2020007483.

Hill, A., & Hill, Q. A. (2018). Autoimmune hemolytic anemia. *Am. Soc. Hematol.*, 382–389. 10.36290/vnl.2018.072.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y.i., Zhang, L.i., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L.i., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q.i., Wang, J., Cao, B., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395 (10223), 497–506.

Jandl, J.H., Kaplan, M.E., 1960. The destruction of red cells by antibodies in man. iii. quantitative factors influencing the patterns of hemolysis in vivo. J. Clin. Investig. 39 (7), 1145. https://doi.org/10.1172/JCI104129.

Jandl, J.H., Jones, A.R., Castle, W.B., 1957. The destruction of red cells by antibodies in man. I. Observations on the sequestration and lysis of red cells altered by immune mechanisms. J. Clin. Investig. 36 (10), 1428–1459.

Janvier, D., Sellami, F., Missud, F., Fenneteau, O., Vilmer, E., Cartron, J., Rohrlich, P., 2002. Severe autoimmune hemolytic anemia caused by a warm IgA autoantibody directed against the third loop of band 3 (RBC anion-exchange protein 1). Transfusion 42 (12), 1547–1552. https://doi.org/10.1046/j.1537-2995.2002.00235.x.

Jawed, M., Hart, E., Saeed, M., 2020. Haemolytic anaemia: a consequence of COVID-19. BMJ Case Reports 13 (12), 10–13. https://doi.org/10.1136/bcr-2020-238118.

Kurlander, R.J., Rosse, W.F., 1979. Monocyte-mediated destruction in the presence of serum of red cells coated with antibody. Blood 54 (5), 1131–1139.

Kuypers, F.A., 2007. Membrane lipid alterations in hemoglobinopathies. Hematology 2007 (1), 68–73. https://doi.org/10.1182/asheducation-2007.1.68.

Lai, M., Leone, G., Landolfi, R., 2013. Autoimmune hemolytic anemia with gel-based immunohematology tests. Am. J. Clin. Pathol. 139 (4), 457–463. https://doi.org/10.1309/AJCPTU9AEQZXVZD4.

Lazarian, G., Quinquenel, A., Bellal, M., Siavellis, J., Jacquy, C., Re, D., Merabet, F., Mekinian, A., Braun, T., Damaj, G., Delmer, A., Cymbalista, F., 2020. Autoimmune haemolytic anaemia associated with COVID-19 infection. Br. J. Haematol. 190 (1), 29–31.

Libregts, S.F., Gutiérrez, L., De Bruin, A.M., Wensveen, F.M., Papadopoulos, P., Van Ijcken, W., Özgür, Z., Philipsen, S., Nolte, M.A., 2011. Chronic IFN-γ production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. Blood 118 (9), 2578–2588. https://doi.org/10.1182/blood-2010-10-315218.

Lopez, C., Kim, J., Pandey, A., Huang, T., DeLoughery, T.G., 2020. Simultaneous onset of COVID-19 and autoimmune haemolytic anaemia. Br. J. Haematol. 190 (1), 31–32. https://doi.org/10.1111/bjh.16786.

Mahmudpour, M., Roozbeh, J., Keshavarz, M., Farrokhi, S., Nabipour, I., 2020. COVID-19 cytokine storm: the anger of inflammation. Cytokine 133, 155151.

MOHAP ICU team. (2020). *Clinical Management of the Critically ill COVID-19 Patient.* United Arab Emirates- Ministry if Health.

Nemeth, E., Ganz, T., 2014. Anemia of inflammation. Hematol. Oncol. Clin. North Am. 28 (4), 671–681.

Noris, M., Remuzzi, G., 2010. Genetics and genetic testing in hemolytic uremic syndrome/thrombotic thrombocytopenic purpura. Semin. Nephrol. 30 (4), 395–408. https://doi.org/10.1016/j.semnephrol.2010.06.006.

Packman, C.H., 2008. Hemolytic anemia due to warm autoantibodies. Blood Rev. 22 (1), 17–31. https://doi.org/10.1016/j.blre.2007.08.001.

Platton, S., Mendes, N., Booth, C., Lancut, J., Lee, K., Regan, F., Green, L., 2021. Positive direct antiglobulin tests in patients with COVID-19. Transfusion 61 (1), 333–334. https://doi.org/10.1111/trf.16156.

Renoux, C., Fort, R., Nader, E., Boisson, C., Joly, P., Stauffer, E., Robert, M., Girard, S., Cibiel, A., Gauthier, A., Connes, P., 2021. Impact of COVID-19 on red blood cell rheology. Br. J. Haematol. 192 (4), e108–e111. https://doi.org/10.1111/bjh.17306.

Sachs, U.J.H., Röder, L., Santoso, S., Bein, G., 2006. Does a negative direct antiglobulin test exclude warm autoimmune haemolytic anaemia? A prospective study of 504 cases. Br. J. Haematol. 132 (5), 655–656. https://doi.org/10.1111/j.1365-2141.2005.05955.x.

Sheng, L., Wang, X., Tang, N., Meng, F., Huang, L., Li, D., 2021. Clinical characteristics of moderate and severe cases with COVID-19 in Wuhan, China: A retrospective study. Clin. Exp. Med. 21 (1), 35–39. https://doi.org/10.1007/s10238-020-00662-z.

Swann, J.W., Koneva, L.A., Regan-Komito, D., Sansom, S.N., Powrie, F., Griseri, T., 2020. IL-33 promotes anemia during chronic inflammation by inhibiting differentiation of erythroid progenitors. J. Exp. Med. 217 (9) https://doi.org/10.1084/jem.20200164.

Thomas, T., Stefanoni, D., Dzieciatkowska, M., Issaian, A., Nemkov, T., Hill, R.C., Francis, R.O., Hudson, K.E., Buehler, P.W., Zimring, J.C., Hod, E.A., Hansen, K.C., Spitalnik, S.L., D'Alessandro, A., 2020. Evidence of structural protein damage and membrane lipid remodeling in red blood cells from COVID-19 patients. J. Proteome Res. 19 (11), 4455–4469. https://doi.org/10.1021/acs.jproteome.0c00606.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., Peng, Z., 2020. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA– J. Am. Med. Assoc. 323 (11), 1061–1069. https://doi.org/10.1001/jama.2020.1585.

Weiss, G., Ganz, T., Goodnough, L.T., 2019. Anemia of inflammation. Blood 133 (1), 40–50. https://doi.org/10.1182/blood-2018-06-856500.

Zagorski, E., Pawar, T., Rahimian, S., Forman, D., 2020. Cold agglutinin autoimmune haemolytic anaemia associated with novel coronavirus (COVID-19). Br. J. Haematol. 190 (4), e183–e184. https://doi.org/10.1111/bjh.16892.

Zantek, N.D., Koepsell, S.A., Tharp, D.R., Cohn, C.S., 2012. The direct antiglobulin test: A critical step in the evaluation of hemolysis. Am. J. Hematol. 87 (7), 707–709. https://doi.org/10.1002/ajh.23218.