Infection and Drug Resistance

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ORIGINAL RESEARCH

# Association Between Rhesus and ABO Blood Group Types and Their Impact on Clinical Outcomes in Critically Ill Patients with COVID-19: A Multi-Center Investigation

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**Background:** There is increasing evidence suggesting that ABO blood type may play a role in the immunopathogenesis of COVID-19 infection. In addition to ABO blood type, the Rhesus (Rh) factor has also been implicated in various disease processes. Therefore, our study aimed to assess the association between both ABO and Rh blood types in critically ill patients with COVID-19 and their clinical outcomes.

**Methods:** A multicenter retrospective cohort study conducted in Saudi Arabia between March 1, 2020, and July 31, 2021, involving adult COVID-19 patients admitted to Intensive Care Units, aimed to explore potential associations between rhesus blood group types (Positive versus Negative) and clinical outcomes. The primary endpoint assessed was the hospital length of stay (LOS). Other endpoints were considered secondary.

**Results:** After propensity score matching (3:1 ratio), 212 patients were included in the final analysis. The hospital length of stay was longer in a negative Rh blood group compared with patients in the Rh-positive group (beta coefficient 0.26 (0.02, 0.51), p = 0.03). However, neither 30-day mortality (HR 0.28; 95% CI 0.47, 1.25, p = 0.28) nor in-hospital mortality (HR 0.74; 95% CI 0.48, 1.14, p = 0.17) reached statistical significance. Additionally, among the different ABO types, the A+ blood group exhibited a higher proportion of thrombosis/infarction and in-hospital mortality (28.1% and 31.2%, respectively).

**Conclusion:** This study highlights the potential impact of blood group type on the prognosis of critically ill patients with COVID-19. It has been observed that patients with a negative Rh blood group type tend to have a longer hospital stay, while their mortality rates and complications during ICU stay are similar to the patients with a Rh-positive group.

Received: 4 November 2023
Accepted: 14 June 2024
Published: 19 July 2024

**Keywords:** rhesus blood group, Blood group, ABO, length of stay, intensive care units, Critically ill, COVID-19, SARS-CoV-2, acute kidney injury, mortality, MV duration

## Introduction

Coronavirus disease (COVID-19) is an exceptionally transmissible viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Growing evidence suggests that ABO blood type may play a role in the immunopathogenesis of COVID-19 infection.[1] Moreover, there is interest in potential risk factors that affect susceptibility to infection and disease progression.[2] To determine the distribution of ABO and RhD blood groups in Saudi blood donors, a systematic literature search was conducted.[3] The study included 32 publications, revealing that most blood donors in Saudi Arabia were male. The most common blood group phenotype among the donors was O (51+8%), followed by A (27+4%), B (18+8%), and AB (4+2%).[3] Additionally, most blood donors were RhD positive (92+2%).[3] The frequencies of ABO and Rh blood groups in this study were similar to global incidence rates, although there were insignificant differences among the various research findings.[3,3]

Several pathophysiological mechanisms were proposed to explain the association between ABO type and COVID-19 infection.[4] One hypothesis suggests that the presence of anti-A and/or anti-B antibodies, particularly in individuals with blood group O, could potentially neutralize the virus by binding to antigens on the viral envelope.[5] Anti-A and anti-B antibodies are specific antibodies produced by the immune system in response to the presence of certain blood group antigens.[5] This interaction may prevent viral infection of target cells.[5] Another hypothesis assumes that the spike (S) proteins of COVID-19 could be bound by naturally occurring anti-A isoagglutinins found in blood group O and B individuals, inhibiting virus-ACE2 receptor interactions.[6] This, in turn, may impede the virus's entry into lung epithelial cells. Furthermore, variations in angiotensin-converting enzyme-1 (ACE1) activity and levels of von Willebrand factor (VWF) and factor VIII, particularly in individuals with blood group A, may contribute to differences in adverse outcomes.[6]

Previous research has shown a potential relationship between ABO blood groups and coagulation abnormalities associated with COVID-19.[7] A multi-institutional data were retrospectively reviewed by Latz et al, reported that patients with COVID-19 and blood group A had a significantly higher risk of developing coagulation abnormalities.[7] These abnormalities can manifest as either excessive clotting (thrombosis) or insufficient clotting (hemorrhage) due to several reasons such as elevated D-dimer levels and prolonged prothrombin time, compared to individuals with blood group O.[7] On the other hand, individuals with blood group O had a lower risk of developing these coagulation abnormalities.[7] These findings suggest that ABO blood groups may play a role in the coagulation abnormalities observed in patients with COVID-19.[7]

Notably, there are conflicting results regarding blood type and COVID-19 infection.[8,9] These contradicting findings in previous studies might be attributed to differences in the studied population (non-critically ill), the comparison groups, the geographical locations, and the confounding factors (age, comorbidities, and using volunteer blood donors as controls).[8,9] In addition, the International Society of Blood Transfusion (ISBT) found no definitive results about the association between blood type and COVID-19 risk and thus recommended that further studies are needed.[4] The majority of the studies showed that patients with blood type A have a higher incidence of COVID-19,[10,11] whereas those with blood type O have a lower risk of infection. However, some studies have different results from the majority.[7,12]

A recent retrospective study examined the health records of patients with COVID-19 who were admitted to the intensive care units (ICUs) in Qatar . The study population was classified based on blood type, including: A, B, AB, O, and Rh (Rh)-positive and Rh-negative. The author reported no association between ABO blood types and adverse clinical outcomes in critically ill patients with COVID-19.[13] However, Rh-negative blood type patients were associated with a lower incidence of death compared to the Rh-positive blood type, who were more prone to COVID-19 complications.[13] The aforementioned study did not investigate other ICU complications such as thrombosis, acute kidney injury (AKI), liver injury, and the development of new-onset atrial fibrillation. Although few studies assessed the associations between blood type and clinical outcomes in patients with COVID-19, some of these studies did not investigate the association

between the Rh blood factor and the clinical outcomes of critically ill COVID-19 patients.[7,10–13] The relationship between blood type and COVID-19 infection has generated conflicting results in previous studies. In addition, it's unclear how the combined effects of ABO and Rh blood types influence the disease course and outcomes in patients diagnosed with COVID-19. Therefore, this retrospective cohort study aims to assess the association between the Rh blood type of critically ill patients with COVID-19 and their clinical outcomes.

# Methods

## Study Design

This research study is related to the Saudi Critical Care Pharmacy Research (SCAPE) platform, which conducted several studies to assess the safety and effectiveness of various treatments for critically ill patients.[14] This multicenter retrospective cohort study was conducted at five centers in the Kingdom of Saudi Arabia (KSA). The study design was chosen to ensure generalizability and increase the study power in estimating the association between Rh blood group and clinical outcomes in critically ill COVID-19 patients. The study involved adult COVID-19 patients admitted to Intensive Care Units (ICUs) between March 1, 2020, and July 31, 2021. All eligible patients were categorized into two sub-groups based on the Rh blood group type (Positive versus Negative). Rh-positive blood group type (Control) is defined as the presence of Rh factor in the blood (ie, O+, A+, AB+, B+), while the absence of Rh factor found in patients with negative Rh blood group type (active) (ie, O-, A-, AB-, B-). No formal treatment pathway was in place for the management of adult COVID-19 patients based on the Rh blood group type (Positive versus Negative). All patients were followed during their hospital stay from the ICU admission date. The study received approval from the King Abdullah International Medical Research Center (KAIMRC) - Institutional Review Board (IRB). Informed consent from the study patients was waived due to the retrospective observational nature of the study.

## Study Setting

This multicenter study was conducted across five medical centers in different geographic distributions within Saudi Arabia. The principal center was King Abdulaziz Medical City, a tertiary care institution located in Riyadh; other centers included were King Abdullah bin Abdulaziz University Hospital (Riyadh), King Abdulaziz University Hospital (Jeddah), King Abdulaziz Medical City (Jeddah), and King Salman Specialist Hospital (Hail).[15–20] The selection of centers was determined by their advanced intensive care unit (ICU) facilities, encompassing both tertiary and secondary hospitals. These facilities were adept at managing critically ill patients diagnosed with COVID-19, employing the standardized national COVID-19 management protocol established by the Ministry of Health (MOH). Factors influencing the choice included geographical distribution, the presence of electronic record-keeping systems, and the centers' expressed willingness to actively participate in the study.

## Study Participants

All adult patients (≥18 years-old) who were critically ill and admitted to the ICUs with confirmed Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Moreover, patients who were designated as "Do-Not-Resuscitate" at admission, had unknown blood group type, died within the first 24 hours of ICU admission, or had an ICU length of stay (LOS) ≤ one day were excluded from our cohort (Figure 1).

## Data Collection

The electronic health records of participating centers were utilized to extract the medical record numbers (MRNs) of critically ill patients admitted to the intensive care unit (ICU) due to COVID-19. Subsequently, a meticulous assessment of patients' eligibility was conducted based on predefined inclusion criteria. Following eligibility confirmation, dedicated teams of co-investigators, assigned from each center, were tasked with inputting the gathered data into the Research Electronic Data Capture (REDCap®) platform hosted by the KAIMRC. Rigorous oversight was maintained throughout the data entry process, with frequent reviews conducted by the research team leader at each participating center to ensure accuracy and uniformity, thereby upholding the overall quality of the collected information. Variables and data collected,

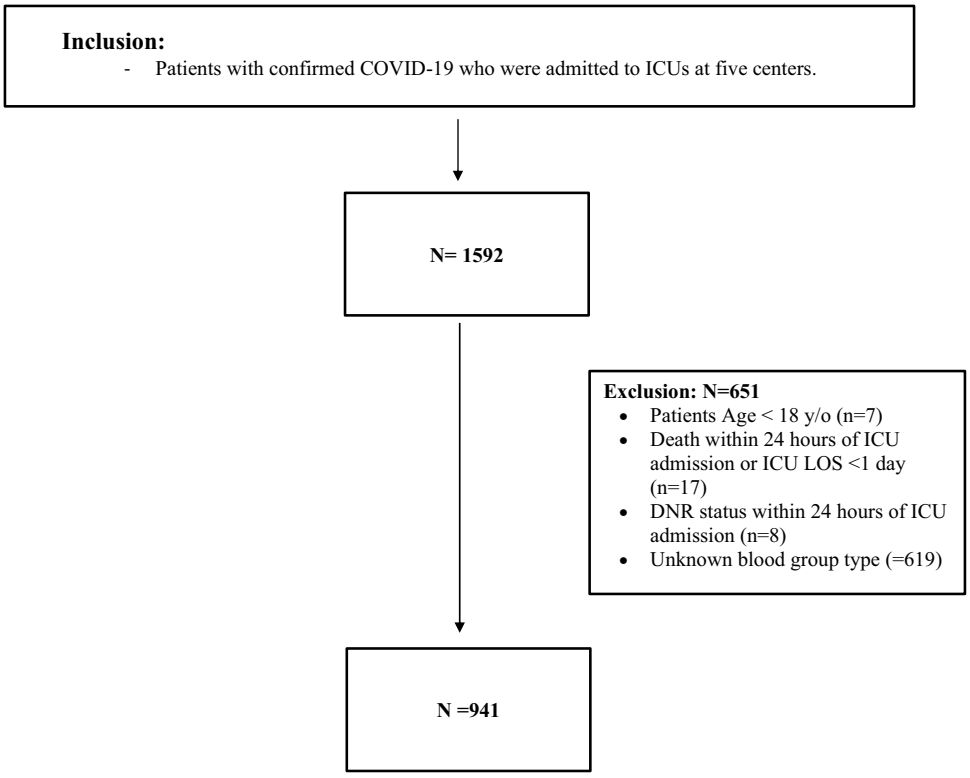


**Figure 1** Flow diagram showing patient recruited with COVID-19.

included but were not limited to demographic data, blood group type, comorbidities, laboratory baseline, severity scores, interventions used, and complications during the ICU stay.

## Outcomes

The primary endpoint was hospital length of stay (LOS). The secondary endpoints were ICU LOS, MV duration, and complications during ICU stay (ie, all thrombosis cases, AKI, liver injury, and new-onset atrial fibrillation) (Outcomes definition – Supplementary file 1).

## Statistical Analysis

The anticipated average hospital length of stay is eight days in the Negative Rh group and 11 days in the Positive Rh group, with an overall standard deviation of 6.5 days. Based on the above assumption, the sample size was calculated to be 200 to provide a power of 90% and an alpha error of less than 5%. We used propensity score (PS) matching to ensure that patients in the active and control groups had similar baseline characteristics. This approach helps minimize the risk of bias and confounding factors affecting the study results. It also helps to ensure that any observed differences in outcomes between the two groups can be attributed to the intervention (active group) being tested rather than other factors.

PS matching using the greedy nearest-neighbor matching method was implemented (Proc PS match) to match one patient with negative Rh blood group (active group) to three patients who had Rh factor in the blood (control group) using 3:1 ratios. Propensity scores were generated after considering all relevant covariates, which included the patient's gender, BMI, baseline APACHE II score, ferritin, and aspartate aminotransferase (AST) levels within 24 hours of ICU admission. Specifically, patients were matched only if the difference in the PS between pairs of patients from the two groups was $\leq 0.1$ times the pooled standard deviation (SD) estimate, which eventually produced the smallest within-pair difference among all available pairs with treated patients. The quality of the matched samples was evaluated by graphing the propensity scores of the two groups. In addition, the Standardized mean difference (SMD) was calculated for the

Negative and Positive Rh blood groups for the PSM cohorts. Our results showed that 1:3 PSM yielded smaller SMD values of 0.1.

Regression analysis such as Cox proportional hazards, logistic, and negative binomial regression analysis were utilized as appropriate. We used Cox proportional hazards regression to examine time-to-event outcomes, which is often suitable for survival analysis. This model allows us to assess the impact of various covariates on the hazard rate, providing valuable insights into the duration until a particular event occurs. The model's validity was assessed using Schoenfeld residuals and likelihood ratio test. Logistic regression was employed for binary outcomes and reported the estimates of odds ratios, allowing us to quantify the relationship between predictor variables and the probability of an event. Negative binomial regression was chosen when dealing with over-dispersed data. There was no imputation for missing data, and SAS software was used for all statistical analyses. A P-value of $< 0.05$ was considered statistically significant for all types of analyses.

## Results

The present investigation enrolled a total of 941 critically ill patients diagnosed with COVID-19 out of 1592 patients who were initially screened. Based on the eligibility criteria, patients were classified into two subgroups; specifically, those exhibiting a Rh-positive blood group type (control) comprising of 849 patients (89.9%) and those with a negative Rh blood group (active), which was discovered in individuals lacking the Rh factor, such as O-, A-, AB-, and B-. The latter group included 95 patients (10.0%). After applying the propensity score matching technique (PS), a 3:1 ratio was used to match the groups, yielding a control group consisting of 159 patients and an active group of 53 individuals, as detailed in Table 1.

### Demographics and Clinical Characteristics

The majority of patients in our cohort were male (61.9%) and had a mean age of 63.0 ± 14.76, with a median BMI of 29.4 (25.59, 34.24). Diabetes mellitus was found to be the most prevalent underlying comorbidity (61.3%), followed by hypertension (60.7%), dyslipidemia (21.1%), and chronic kidney disease (15.5%). Prior to the application of PS matching, noted differences between the Rh blood group types were observed, where the Rh-positive blood group was primarily composed of individuals with O+ (44.2%), followed by A+, B+, and AB+ (26.1%, 19.5%, 4.5%, respectively). In contrast, the negative group predominantly consisted of patients with O- (4.0%), followed by A-, B-, and AB- (3.3%, 2.4%, 0.4%, respectively). Upon the implementation of PS matching, most of these differences were less pronounced between the two groups (Tables 1 and 2).

### Length of Stay and MV Duration

A significant difference in hospital length of stay was observed between two groups, with longer durations in patients with Rh-negative blood group compared to those with Rh-positive blood group (beta coefficient 0.26, $p = 0.04$). On the other hand, the ICU length of stay in both groups was comparable and not-statically significant (beta coefficient 0.13 (−0.12,0.37), $p = 0.32$). Additionally, in the crude analysis, no significant difference was found MV duration between the two groups, with a comparable average of 12 days versus 13.5 days ($p = 0.39$). After undergoing regression analysis, the results remained non-statistically significant with a beta coefficient (95% CI) of 0.24 (−0.10, 0.58) and a P-value of 0.16 (Table 3).

### Mortality

The in-hospital Mortality rate was insignificantly higher in Rh-positive compared to Rh-negative patients. Furthermore, the ABO types, A+, O+, and followed by B+, were the most common blood group types with higher proportions (31.2%, 26.6%, and 16.4% respectively). In addition, using cox regression analysis, neither 30-day mortality (HR 0.28; 95% CI 0.47, 1.25, $p = 0.28$) nor in-hospital mortality (HR 0.74; 95% CI 0.48, 1.14, $p = 0.17$) reached statistical significance (Table 3).

**Table 1** Summary of Demography and Baseline Characteristics

| | Before Matching | | | | After Matching | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable (s)** | **Overall (N=941)** | **Positive (N=846)** | **Negative (N=95)** | **P-value** | **Overall (N=212)** | **Positive (N=159)** | **Negative (N=53)** | **P-value** |
| Age (Years), Mean (SD) | 63.0 (14.76) | 63.0 (14.63) | 62.5 (15.93) | 0.8790^ | 63.8 (14.68) | 64.2 (14.66) | 62.8 (14.80) | 0.5532^ |
| Gender – Male, n (%) | 566 (61.9) | 517 (62.9) | 49 (52.7) | 0.0548^^ | 114 (54.0) | 83 (52.5) | 31 (58.5) | 0.4513^^ |
| BMI, Median (Q1,Q3) | 29.4 (25.59, 34.24) | 29.4 (25.47, 34.21) | 31.2 (26.40, 34.60) | 0.2162^ | 30.1 (25.39, 34.24) | 30.0 (25.59, 33.91) | 31.2 (24.69, 35.49) | 0.6917^ |
| Blood group type, n (%) | | | | | | | | |
| A+ | 240 (26.1) | 240 (29.1) | 0 (0.0) | <0.0001** | 52 (24.6) | 52 (32.9) | 0 (0.0) | <0.0001** |
| B+ | 179 (19.5) | 179 (21.7) | 0 (0.0) | <0.0001** | 33 (15.6) | 33 (20.9) | 0 (0.0) | |
| O+ | 365 (39.8) | 365 (44.2) | 0 (0.0) | <0.0001** | 62 (29.4) | 62 (39.2) | 0 (0.0) | |
| AB+ | 41 (4.5) | 41 (5.0) | 0 (0.0) | <0.0001** | 11 (5.2) | 11 (7.0) | 0 (0.0) | |
| A- | 30 (3.3) | 0 (0.0) | 30 (32.3) | <0.0001** | 17 (8.1) | 0 (0.0) | 17 (32.1) | |
| B- | 22 (2.4) | 0 (0.0) | 22 (23.7) | <0.0001** | 14 (6.6) | 0 (0.0) | 14 (26.4) | |
| O- | 37 (4.0) | 0 (0.0) | 37 (39.8) | <0.0001** | 21 (10.0) | 0 (0.0) | 21 (39.6) | |
| AB- | 4 (0.4) | 0 (0.0) | 4 (4.3) | <0.0001** | 1 (0.5) | 0 (0.0) | 1 (1.9) | |
| APACHE II score, Median (Q1,Q3) | 15.0 (11.00, 24.00) | 15.0 (10.00, 23.00) | 17.0 (11.00, 26.00) | 0.1230^ | 16.0 (11.00, 25.00) | 16.0 (11.00, 24.00) | 15.0 (11.00, 26.00) | 0.7303^ |
| SOFA score, Median (Q1,Q3) | 5.0 (3.00, 8.00) | 5.0 (3.00, 8.00) | 5.0 (3.00, 8.00) | 0.8637^ | 5.0 (3.00, 8.00) | 5.0 (3.00, 7.00) | 5.0 (2.00, 8.00) | 0.6897^ |
| Early use of Dexamethasone within 24 hours, n (%) | 563 (61.3) | 507 (61.5) | 56 (60.2) | 0.8160^^ | 124 (58.8) | 94 (59.5) | 30 (56.6) | 0.7115^^ |
| Early use of Tocilizumab within 24 hours, n (%) | 166 (18.1) | 151 (18.3) | 15 (16.1) | 0.6056^^ | 30 (14.2) | 22 (13.9) | 8 (15.1) | 0.8328^^ |
| Serum creatinine (mg/dL) at admission, Median (Q1,Q3) | 1.1 (0.78, 1.65) | 1.1 (0.78, 1.63) | 1.1 (0.74, 1.67) | 0.4796^ | 1.1 (0.76, 1.57) | 1.1 (0.76, 1.57) | 1.0 (0.76, 1.60) | 0.5223^ |
| Blood Urea nitrogen (BUN) (mg/dL) at admission, Median (Q1,Q3) | 8.0 (5.00, 13.50) | 8.0 (5.19, 13.44) | 8.0 (4.75, 15.30) | 0.7639^ | 8.1 (5.00, 14.00) | 8.2 (5.06, 13.75) | 8.0 (4.80, 14.30) | 0.7094^ |
| Oxygenation Index (OI), Median (Q1,Q3) | 16.1 (8.12, 25.95) | 16.1 (8.27, 26.02) | 15.2 (7.10, 24.28) | 0.3521^ | 13.0 (7.02, 25.48) | 13.1 (6.94, 26.30) | 10.8 (7.10, 24.81) | 0.6364^ |
| Lactic acid (mmol/L) Baseline, Median (Q1,Q3) | 1.7 (1.22, 2.40) | 1.7 (1.22, 2.46) | 1.7 (1.19, 2.24) | 0.6860^ | 1.5 (1.10, 2.08) | 1.4 (1.10, 1.97) | 1.7 (1.14, 2.24) | 0.1375^ |
| Platelets count (10^9/L) Baseline, Median (Q1,Q3) | 238.0 (184.00, 312.00) | 238.0 (184.00, 312.00) | 238.5 (185.50, 326.50) | 0.5810^ | 252.0 (191.00, 333.00) | 255.0 (193.00, 328.00) | 245.5 (177.50, 354.50) | 0.9894^ |
| Total WBC Baseline, Median (Q1,Q3) | 9.6 (6.53, 13.50) | 9.6 (6.53, 13.30) | 10.5 (6.71, 14.60) | 0.1583^ | 10.1 (7.57, 14.00) | 9.8 (7.57, 14.00) | 11.5 (7.70, 15.15) | 0.2625^ |
| International normalized ratio (INR), Median (Q1,Q3) | 1.1 (1.02, 1.23) | 1.1 (1.02, 1.23) | 1.1 (1.01, 1.20) | 0.4184^ | 1.1 (1.02, 1.23) | 1.1 (1.01, 1.23) | 1.1 (1.03, 1.21) | 0.8599^ |
| C-reactive protein (CRP) baseline (mg/l), Median (Q1,Q3) | 130.0 (68.50, 201.00) | 129.0 (69.80, 197.00) | 136.0 (63.00, 220.00) | 0.9431^ | 125.0 (75.00, 195.00) | 120.0 (77.00, 182.00) | 135.0 (63.00, 211.00) | 0.9001^ |
| D-dimer (mg/l) Level baseline, Median (Q1,Q3) | 1.5 (0.81, 3.69) | 1.5 (0.80, 3.76) | 1.2 (0.85, 3.29) | 0.3856^ | 1.4 (0.77, 3.39) | 1.5 (0.77, 3.05) | 1.3 (0.77, 3.70) | 0.8973^ |
| Ferritin Level (ug/l) baseline, Median (Q1,Q3) | 662.0 (333.00, 1584.00) | 680.0 (338.40, 1610.00) | 540.1 (276.90, 1076.00) | 0.1158^ | 532.1 (286.00, 1038.00) | 533.4 (288.10, 992.60) | 507.5 (243.60, 1076.00) | 0.9265^ |
| Lowest PaO2/FiO2 ratio within 24 hours of admission, Median (Q1,Q3) | 83.8 (60.28, 143.90) | 84.0 (60.40, 143.30) | 82.5 (57.77, 163.80) | 0.9007^ | 86.2 (60.00, 154.00) | 86.3 (60.46, 151.50) | 80.7 (55.90, 163.80) | 0.6330^ |

**Notes**: *t-Test / ^ Wilcoxon rank sum test is used to calculate the P-value. ^^ Chi-square test / Fisher exact test is used to calculate the P-value.

**Table 2** Continue Summary of Demography and Baseline Characteristics

| Variable (s) | Before Matching | | | | After Matching | | | |
|---|---|---|---|---|---|---|---|---|
| | Overall (N=941) | Positive (N=846) | Negative (N=95) | P-value | Overall (N=212) | Positive (N=159) | Negative (N=53) | P-value |
| **Comorbidities** | | | | | | | | |
| Hypertension | 557 (60.7) | 505 (61.2) | 52 (55.9) | 0.3214^^ | 140 (66.4) | 107 (67.7) | 33 (62.3) | 0.4669^^ |
| Diabetes Mellitus | 563 (61.3) | 507 (61.5) | 56 (60.2) | 0.8160^^ | 138 (65.4) | 107 (67.7) | 31 (58.5) | 0.2215^^ |
| Dyslipidemia | 194 (21.1) | 179 (21.7) | 15 (16.1) | 0.2125^^ | 47 (22.3) | 42 (26.6) | 5 (9.4) | 0.0094^^ |
| Heart Failure | 91 (9.9) | 83 (10.1) | 8 (8.6) | 0.6555^^ | 23 (10.9) | 20 (12.7) | 3 (5.7) | 0.1572^^ |
| Asthma | 60 (6.5) | 54 (6.5) | 6 (6.5) | 0.9723^^ | 8 (3.8) | 5 (3.2) | 3 (5.7) | 0.4104** |
| COPD | 24 (2.6) | 23 (2.8) | 1 (1.1) | 0.3265** | 10 (4.7) | 10 (6.3) | 0 (0.0) | 0.0606** |
| Chronic kidney disease (CKD) | 142 (15.5) | 129 (15.6) | 13 (14.0) | 0.6751^^ | 33 (15.6) | 29 (18.4) | 4 (7.5) | 0.0609^^ |
| Cancer | 62 (6.8) | 55 (6.7) | 7 (7.5) | 0.7540^^ | 10 (4.7) | 7 (4.4) | 3 (5.7) | 0.7153** |
| Liver disease (any type) | 26 (2.8) | 26 (3.2) | 0 (0.0) | 0.0824** | 5 (2.4) | 5 (3.2) | 0 (0.0) | 0.1900** |

**Notes**: *t-Test / ^ Wilcoxon rank sum test is used to calculate the P-value. ^^ Chi-square test / Fisher exact test is used to calculate the P-value.

**Table 3** Clinical Outcomes of Critically Ill Patients with COVID-19 After Matching

| Outcomes∆ | Rhesus Blood Group | | P-value | Hazard Ratio (HR) (95% CI) | P-value $* |
|---|---|---|---|---|---|
| | Positive | Negative | | | |
| **30-day mortality, n (%)** | 79 (51.0) | 21 (42.9) | 0.32^^ | 0.28 (0.47, 1.25) | 0.28 |
| **In-hospital mortality, n (%)** | 100 (64.1) | 27 (54.0) | 0.20^^ | 0.74 (0.48, 1.14) | 0.17 |
| | | | | **Beta coefficient (Estimates) (95% CI)** | **P-value $**** |
| **MV duration (Days), Median (Q1,Q3)** | 12.0 (5.00, 20.00) | 13.5 (5.00, 26.00) | 0.39^ | 0.24 (−0.10,0.58) | 0.16 |
| **ICU Length of Stay (Days), Median (Q1,Q3)** | 14.5 (8.00, 21.00) | 14.5 (8.50, 27.00) | 0.46^ | 0.13 (−0.12,0.37) | 0.32 |
| **Hospital Length of Stay (Days), Median (Q1,Q3)** | 20.0 (14.00, 28.00) | 24.0 (13.00, 34.00) | 0.28^ | 0.26 (0.02,0.51) | 0.04 |

**Notes**: ∆Denominator of the percentage is the total number of patients. *t-Test / ^ Wilcoxon rank sum test is used to calculate the P-value. ^^ Chi-square test / Fisher exact test is used to calculate the P-value. $* Cox proportional hazards regression analysis used to calculate HR and p-value. $** Generalized linear model is used to calculate estimates and p-value.

## Complication (s) During ICU Stay

All thrombosis cases between the two groups was comparable (OR (95% CI) = 1.68 (0.75,3.78), p = 0.21). Among the ABO types, the A+ blood group type had a higher proportion of thrombosis and infarction (28.1%). Moreover, there was no statistically significant difference in the occurrence of complications during ICU stay between the two groups in the crude analysis as well in regression analysis, such as acute kidney injury (AKI) (OR (95% CI) = 1.47 (0.78,2.77), p = 0.23), liver injury (OR (95% CI) = 0.73 (0.19, 2.72), p = 0.64), and new onset Afib (OR (95% CI) = 1.06 (0.42, 2.66), p = 0.91) (Table 4).

**Table 4** Complications During ICU After PS Matching and Multivariable Regression

| Outcomes∆ | Rhesus Blood Group | | P-value | Odds Ratio (OR) (95% CI) | P-value $ |
|---|---|---|---|---|---|
| | Positive | Negative | | | |
| **Acute kidney injury, n (%)** | 75 (47.5) | 30 (56.6) | 0.25^^ | 1.47 (0.78,2.77) | 0.23 |
| **New onset A fib, n (%)** | 19 (12.0) | 7 (13.2) | 0.82^^ | 1.06 (0.42,2.66) | 0.91 |
| **Liver injury, n (%)** | 12 (7.6) | 3 (5.7) | 0.63** | 0.73 (0.19,2.72) | 0.64 |
| **All thrombosis cases, n (%)** | 21 (13.5) | 11 (20.8) | 0.21^^ | 1.68 (0.75,3.78) | 0.21 |

**Notes**: ∆ Denominator of the percentage is the total number of patients. *t-Test / ^ Wilcoxon rank sum test is used to calculate the P-value. ^^ Chi-square test / Fisher exact test is used to calculate the P-value. $ Logistic regression is used to calculate the OR and p-value.

## Discussion

This study was conducted to identify the association between the Rh blood group and the clinical outcomes in critically ill patients diagnosed with COVID-19. Several existing risk factors can increase the susceptibility to COVID-19 infection and worsen clinical impacts in critically ill patients.[11,13] Evidence that supports the association between the Rh blood group and COVID-19 infection in critically ill patients is not well established. The magnitude of the COVID-19 predisposition between two phenotype groups was reported inconsistently in earlier studies.[21–25] Previous studies have observed a protective effect among Rh-negative blood patients for intubation and mortality and a 2.7% lower risk of initial infection.[12,13,21,26–28] The results from our study have provided insight into the neutral effect of the Rh-blood group on ICU mortality, duration of mechanical ventilation, ICU length of stay, and complications during ICU stay in critically ill patients with COVID-19.

The most important finding of our study is the association of the negative blood group with a 4-day increase in hospital length of stay compared to the Rh-positive blood group. Consistent with previous research, hospital length of stay was reported to be higher in the Rh-negative group.[28] The increased hospital LOS is a novel finding in this patient population. Previously reported studies did not show any association between Rh-blood groups and hospital length of stay.[11,13,24,27]

The secondary endpoint of our study was to determine if the Rh-blood group impacted ICU LOS and clinical outcomes. We analyzed ICU LOS among our cohort and observed no difference in ICU LOS, although our sample size is insufficient to detect a difference. Multiple subsequent studies reported similar findings between Rh-positive and Rh-negative blood groups.[7,11,13,22,27]

Yaylaci et al investigated the rate of ABO and Rh blood types in COVID-19 patients and the relationships of these frequencies with ICU admissions. They did not differentiate between ICU length of stay between the two groups. Additionally, the included patients were analyzed to be similar in at least one chronic disease among different Rh blood types, allowing for multiple other comorbidities possibly explaining the higher observed rates of ICU admission among the Rh-positive blood group. Although all patients were classified based on their blood groups, neither comparison analysis of O, A, B, and AB groups with other blood groups revealed a significant relationship with ICU admission.[29] Moreover, Although the study primarily focused on comparing RH-factor, patients with A blood type showed the highest percentage of thrombosis/ infarction incidence (28.13%). This could be linked to the fact that blood group O individuals have lower plasma levels of procoagulant factor VIII and Von Willebrand factor than other blood groups. Additionally, previous studies showed that A blood type patients tend to have more severe complications from COVID-19. In summary, our results appear to confirm the results of a multi-center retrospective study demonstrated that for different Rh-blood types, the duration in the ICU does not confer any differences.[11]

Although Rh-positive blood group patients exhibited a higher rate of 30-day mortality and hospital mortality than the Rh-negative group, the differences were not statistically significant. The results of this outcome appear to confirm multiple previous studies demonstrating that all-cause ICU mortality rates were higher in Rh-positive than the Rh-negative group.[11,13,26] A combined category of patients with any risk factor for mortality was included in the Cox regression analysis and determined to be non-significant. In a large single-center retrospective study, the reported

mortality rate was higher among the Rh-negative group. After adjusting for possible confounders, the mortality difference was found to be non-significant between the two groups.[30] Three subsequent studies showed no relevant association between the Rh group and mortality.[7,11,27–29,31] The difference in findings can likely be attributed to the larger sample size and the inclusion of hospital records spanning 30 days. Given the prevalence of underlying comorbidities among nearly all patients, it remains unclear whether distinguishing between blood types would confer any survival advantage.

The authors acknowledge limitations in this analysis. The study primarily relied on a retrospective design, focusing on a pragmatic approach to include COVID-19 patients. Documentation from healthcare providers and the use of two different testing methods (RT-PCR and throat swabs) could influence initial treatment decisions and disease progression assessments. A higher number of patients assigned to the Rh-positive group suggests a normal blood type distribution in the Kingdom of Saudi Arabia.[32,33] During the study period, the Saudi Ministry of Health protocol for COVID-19 patients changed, suggesting differences in testing or workup for alternative diagnoses could affect the study outcomes. In this study, we did not assess outcomes by specific ABO blood groups. We may have overlooked subtle but potentially significant variations in disease impact and patient response to treatment. We suggest that future research should include a detailed analysis of ABO blood group subtypes to enhance the applicability of findings to targeted therapeutic interventions and risk stratification.

Additionally, we could not account for other factors, such as the onset of COVID-19 symptoms, that may affect the decision to offer initial therapy in the emergency department. Although prior studies demonstrated ABO blood group types are associated with increased severity of infections, we did not assess the clinical outcomes or the cost and time associated with specific Rh blood groups. The rationale for differentiating blood types among COVID-19 patients is likely multifactorial, including a perceived medical/medication history, onset of COVID-19 symptoms, initial selected therapy for infected patients, or disease progression among hospitalized patients.

## Conclusion

In Conclusion, our study suggests that critically ill patients with COVID-19 and negative Rh blood group may experience longer hospital stays. Early recognition of these individuals, based on their blood type or Rh blood group, may be crucial in implementing prompt measures to control the infection, prevent complications, and minimize the financial impact on the healthcare system.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethical Approval and Informed Consent Statement

The study protocol was reviewed and approved by the Institutional Review Board of King Abdullah International Medical Research Center (IRB-KAIMRC), Riyadh, Saudi Arabia. Obtaining consent was waived by the IRB-KAIMRC due to the retrospective nature of the study. The study was conducted in accordance with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (adopted 1964; updated 2013), national ethical regulations, and local institutional guidance of study centers.

## Acknowledgments

We would like to thank all the investigators who participated in this project from the Saudi Critical Care Pharmacy Research (SCAPE) platform. We express our appreciation to all researchers affiliated with the Saudi Critical Care Pharmacy Research (SCAPE) platform, as well as the supporters from the Saudi Society for Multidisciplinary Research Development and Education, for their invaluable assistance in this project. Also, we would like to acknowledge Dr. Sama Al Shujairi for help in the introduction section drafting. The authors would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

All authors declare no conflicts of interest in this work.

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