Editorial

A Caution in Association of ABO Blood Group with COVID-19

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The coronavirus disease 2019 (COVID-19) pandemic has evoked unprecedented intensive research efforts by biomedical scientists and physicians in a wide range of research and healthcare institutions. These efforts have led to an increased number of scientific papers and manuscripts, which are beginning to shape our understanding of the virus and its genomic structure, patterns of pathophysiology, clinical manifestation and features, options for therapeutic development and management, as well as preventative health measures that have already been implemented or are foreseeable. In this fast-paced research and reporting milieu, a concern is that suitable rigor of study must be applied, so that sound scientific and medical conclusions can be reached. An appropriate part of the process that makes discoveries accessible as soon as possible has proven to be that of researchers uploading their manuscripts onto the preprint servers for preliminary publications, so that observations can reach a broad readership even when the peer-review process has not been completed. Nonetheless, some of the many manuscripts without peer-review may have caused confusion or triggered controversy due to inadequate attention to a rigorous design at the outset, which may lead to inconsistent replication of the results. The flood of fast-paced publications, including manuscripts on the preprint servers, has already caused a concern in the society (1). As an illustration, in this commentary, we will address some aspects of needed scientific rigor regarding ABO blood biomarkers for COVID-19 susceptibility, which is determined by the infectious agent, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and host factors that may modify individual's clinical course of COVID-19 after being infected.

We wish to suggest that some instances of insufficient rigor may have occurred in some studies that aimed to identify the ABO blood group as a biomarker for susceptibility to COVID-19. While these manuscripts are still under peer-review, the findings have already been conveyed to the biomedical and scientific community and the lay public. Three studies with hospital-based case series and controls from the general populations (2-4) are of concern in term of the study design. Because exposure to the viral pathogen (SARS-CoV-2) is the first required condition to be infected and then develop the disease, using individuals without clear status of confirmed exposure to the pathogen seems to be a problem. For a straightforward case-control design, the appropriate study population for COVID19 susceptibility should include individuals who have had adequate exposure to confirmed sources of infection, and strictly speaking, had such exposure without using personal protective equipment. In addition, the statistical analyses used in the studies were not standard methods in epidemiology. The odds ratio (OR) calculated for each blood group with references to all alternative blood groups may have caused some problems of comparison and multiple testing. Even so, one study with three case-control samples (2) showed that ABO blood group A was associated with an increased likelihood of developing COVID-19 (OR=1.28; $p<0.001$) in one cohort of 3493 cases and 1888 controls in which the large sample size might overstate the statistical significance. The findings from the other two cohorts within the same study were not significant (OR=1.40) even when the effect size was bigger or completely non-replicable (OR=1). Of note, the OR may not be a viable choice of effect size (5), especially for measuring the relative risk when the event of interest (e.g., blood group A) in the control group is not a rare event.

Similarly, another study (3) found that ABO blood group A was associated with mild (OR=1.40; $p=0.045$) and severe patients (OR=1.63; $p=0.015$), but these findings may not survive the multiple testing entirely. A further study in the US, with a similar study design using general population samples as controls (4), showed that only ABO blood group A with Rh-positive was associated with more likelihood of COVID-19 (OR=1.38, $p=0.004$). However, the association of blood group A with COVID-19 was not significant in patients who were Rh-negative. Also, on June 2, 2020, a genome-wide association study (6), with the similar study design using COVID-19 with respiratory failure as cases and controls derived from general populations, reported a single nucleotide polymorphism rs657152 at ABO gene at 9q34.2 associated with the COVID-19 with respiratory failure in Italy and Spain, the European epicenters for COVID-19. A great effort has been made by the investigators to conduct such a genome-wide association study within a

short time period. These studies might need further statistical analyses to rule out the potential confounding effect by clinical subtypes and underlying chronic health conditions such as cardiovascular diseases and diabetes, which are quite frequent (21-39%) in the hospital-based patients with COVID-19 (7). It would also be of interest to examine the genetic variants associated with clinical subtypes within this data.

We are concerned that the association of ABO blood group and COVID-19 might be at least partially confounded by the comorbidity of cardiovascular disease prevalent in the hospital-based cases, particularly in the severe cases (7). Beyond the role in immunohematology that involves both red blood cell and serum or plasma (**Box 1**), ABO blood group A has been associated with increased risk for chronic diseases, including coronary heart disease (CHD), venous deep thromboembolism (VTE) and gastric cancer (8). A meta-analysis of 17 studies with 225,810 subjects indicated that individuals with ABO blood group A were more likely to have CHD (OR=1.14; p=0.01)(9), probably due to the elevated levels of von Willebrand factor glycoprotein (10), binding to factor VIII, an essential blood-clotting protein. Additionally, genome-wide association studies have found that genetic variants at ABO loci are associated with elevated levels of plasma lipids and inflammatory markers such as the intercellular adhesion molecule 1 (11-13), which may contribute to the risk of cardiovascular diseases (14, 15).

Box 1. ABO blood group, antigen, antibody, and genotype

Blood group	An antigen present on red blood cells	An antibody present in serum	Genotype
A	Antigen A	Anti-B	AA and AO
B	Antigen B	Anti-A	BB and BO
AB	Antigen A; Antigen B	None	AB
O	None	Anti-A; Anti-B	OO

Other observations show that ABO blood group has an association with some infectious diseases or conditions but in opposing direction of effect. For example, ABO blood group O is associated with the risk of having cholera, *Helicobacter pylori,* and norovirus infections;(16), whereas the non-O group is associated with the risk of severe malaria (17). ABO blood group is also heterogeneous across the world populations (4). Of note, ABO typing can be affected by conditions such as genetic variation (secretor/FUT2), developmental stage, and other diseases, and therefore, cannot always be ascertained by red blood cell type alone (16). These aspects require additional cautions for the study of ABO and COVID19, in particular using a case-control design.

Furthermore, the molecular and cellular components of the cardiovascular system may mediate the susceptibility to infections. The virus SARS-CoV2 is known to be the causative factor of COVID-19, and its genome encodes for four structural proteins (S, E, M, and N) that form the outer layer for protecting the RNA inside. The Spike protein (S) appears optimized for binding to multiple human receptors (18), including the angiotensin-converting enzyme 2 (ACE2) (19), which serves as the entry point of the virus SARS-CoV-2 onto the human cells. ACE2 which is highly expressed in the epithelial cells of the lung, intestine, kidney, heart, and the endothelium of blood vessels (20), is a vasopeptidase that plays a role in cardiovascular disease and the immune system (21). Therefore, the increased levels of ACE2 expression, which could be modulated by the treatment with ACE inhibitors (ACEIs)/angiotensin receptors blockers (ARBs) (22) or genetic variation (23) in the key molecules, may increase the likelihood of being infected after individuals are exposed to the viral pathogen. In addition, COVID-19 is prevalently comorbid with hypertension, coronary heart disease, and diabetes. There is a concern whether the ACEIs/ARBs should continue to be used to treat hypertension and related conditions during the ongoing pandemic of COVID-19. Therefore, individuals with underlying conditions or taking the ACEIs/ARBS should be strongly encouraged to take careful measures to minimize the exposure to source of infection.

In summary, we suggest investigating further the association of ABO and COVID19 with a rigorous approach to reduce the possible confounding effect by the underlying cardiovascular and metabolic conditions, which are known to be associated with both ABO blood group and over-presentation in individuals with COVID-19.

CONFLICT OF INTERESTS

The authors declare no conflict of interest regarding the publication of this paper.

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