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Higher levels of von Willebrand Factor in hospitalised patient plasma provides an explanation for the association of ABO blood group and secretor status with COVID19 severity.

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Conflict of Interest

The authors have disclosed no conflicts of interest.

Early studies of COVID19 in Wuhan suggested a relationship between SARS-CoV-2 infection and an individual's ABO blood group with a higher frequency of infected blood group A patients than would be expected if ABO group had no influence on infection status.[1] Since then numerous additional studies have corroborated this observation, whilst some have found no link at all. [2-4] Possible explanations for this discrepancy may be differences in the frequency of different blood groups within different populations and the presence of underlying disease in infected hospitalised patients when compared with healthy individuals. In a study of COVID19 patients in Bristol, UK, we observed a significant association between COVID19 hospitalisation, blood group A patients who are secretors and cardiovascular complications.[5] Other studies have also reported an association between blood groups and COVID19.[6] There is a well described link between blood group "non-O" (A, B, AB) and thrombotic events centred on higher circulating levels of von Willebrand factor (VWF) in non-O individuals when compared with those of group O.[7] This may be because additional glycosylation of an H antigen, spatially close to amino-acid 1574 within VWF, to form either the A or B antigen, provides protection against proteolytic degradation by the VWF regulator protease ADAMTS13.[7] Fucosylation of a terminal galactose to form the H-antigen on secreted proteins is performed by Fucosyltransferase 2 (FUT2) and individuals with a functional *FUT2* gene express ABO on non-haematological cells and on secreted proteins (such as VWF) and are termed secretors. However, approximately 20% of the population have an inactivating mutation in *FUT2*, consequently they only express ABO on haematological cells, and are termed non-secretors. VWF levels are also known to be higher in group A secretors than group A non-secretors.[8] In addition, patients with COVID19 are known to have higher levels of VWF than those without infection.[9] We set out to explore the relationship between blood groups, secretor status and VWF in relation to COVID19 severity. Samples from individuals hospitalised for COVID19, who had a positive polymerase chain reaction result for SARS-CoV-2 using the established Public Health England reverse transcriptase PCR (RT-PCR) assay, were selected on the basis that a historical plasma sample was available to test and that the patients had been typed for ABO and secretor status. We also tested 84 NHS Blood & Transplant convalescent plasma samples taken at least 28 days after infection as controls. All samples were available from our previous blood group study.[5] We quantified VWF by ELISA in plasma samples from 66 hospitalised patients with ongoing active COVID19 and of known ABO group (from the DISCOVER cohort[5]) and found significantly higher levels of VWF when compared with plasma samples from 84

separate convalescing patients, taken at least 28 days after infection, who had recovered from COVID19 10.8μg/ml c.f. 3.3μg/ml respectively (P<0.0001 (Figure 1A)). VWF levels were highest in hospitalised group A individuals (11.6μg/ml) and significantly higher than patients who had recovered (P<0.0001 (Figure 1B)). These levels remained higher in group A patients following recovery, albeit at much lower levels (3.8μg/ml c.f. 2.8μg/ml in group O, P=0.03, Figure 1B). When factoring secretor status in hospitalised patients, secretors had higher VWF levels than non-secretors in both blood groups, with the highest levels observed in group A secretors (12.2μg/ml, Figure 1C)). VWF levels in hospitalised secretors were significantly higher than all patients who had recovered from COVID19 (P<0.0001, Figure 1C). COVID19 causes inflammatory lung injury and these data suggest that this inflammatory response, or another aspect of COVID19 infection, results in elevated VWF levels which amplifies a pre-existing enhanced susceptibility to thrombosis in secretors compared to non-secretors particularly in "non-O group" individuals. This situation is further exacerbated by the reduction in ADAMTS13 observed in the plasma of patients with COVID19.[9] Our previous study also provided evidence that group A individuals with a functioning FUT2 secretor gene are at greater risk of severe disease than those lacking a functional secretor gene[5] and recent large-scale genomic studies support this conclusion.[10] Our current study suggests that the link between an individual's ABO blood group, secretor status, and susceptibility to develop severe COVID19 is, at least in part, related to the effects that both blood group and SARS-CoV-2 infection have on the circulating levels of VWF and merits further investigation in a larger cohort.

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dedicate this manuscript to the memory of Professor David Anstee who, early in the pandemic (February 2020), had the foresight to initiate our investigation into secretor status alongside ABO group in association with COVID19 disease severity[5] and who then went on to make the link with VWF that led to this study. Although Prof Anstee approved the content presented in the manuscript, he sadly died before submission.

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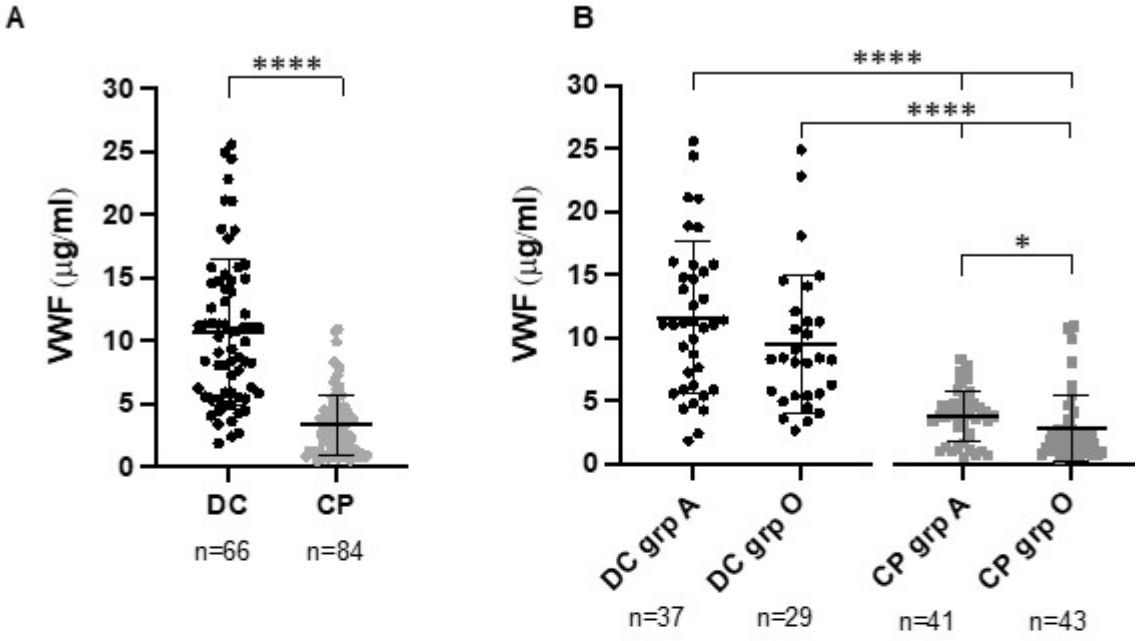
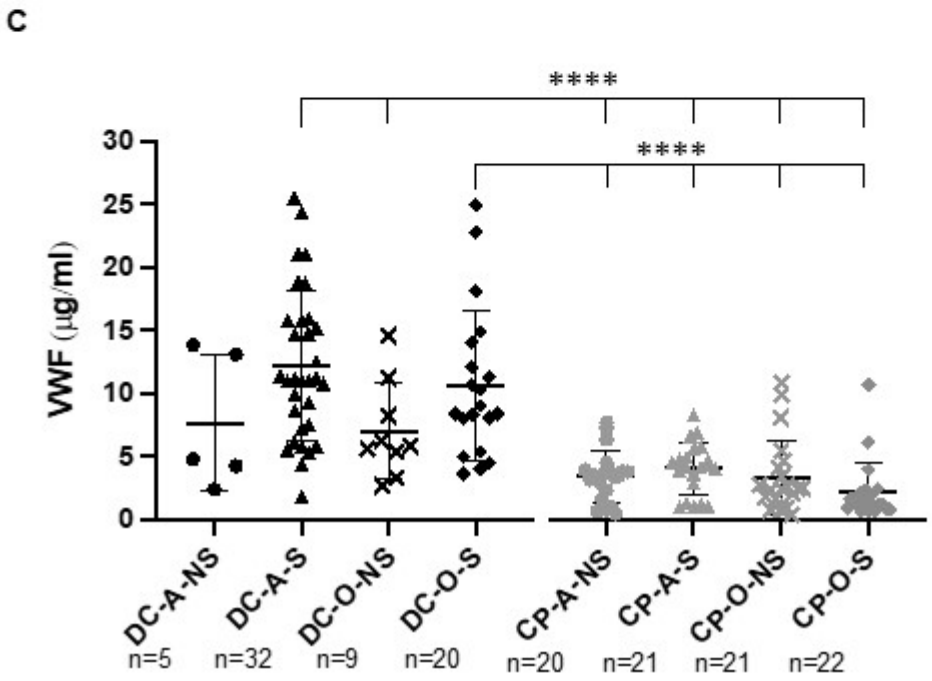
Figure 1 VWF plasma levels in patients hospitalised with active COVID19 or patients who have recovered from COVID19. VWF levels in samples from hospitalised COVID19 patients, from the DISCOVER cohort[5] (DC), and patients who had recovered from COVID19 (CP) were detected by ELISA using a human VWF ELISA Kit (AbCAM, UK) as per the manufacturer's instructions (**A**). VWF levels in hospitalised (DC) and recovered (CP) cases sorted by blood group (A or O) (**B**). Patient samples were further stratified according to secretor status (secretor = S or non-secretor = NS), A or O blood group and disease status (**C**). Each symbol represents the mean value of at least duplicate readings from an individual patient sample. Lines represent mean ±SD for each subgroup. Statistical comparisons were conducted using one tailed T- tests (**A**) or by analyses of variance with Tukey post-hoc testing (B & **C**). *, P<0.05; ****, P<0.0001.


A
B
****
****
****
*
VWF (µg/ml)
30
25
20
15
10
5
0
DC
CP
n=66
n=84
DC grp A
DC grp O
CP grp A
CP grp O
n=37
n=29
n=41
n=43


C
****
****
VWF (µg/ml)
30
25
20
15
10
5
0
DC-A-NS
DC-A-S
DC-O-NS
DC-O-S
CP-A-NS
CP-A-S
CP-O-NS
CP-O-S
n=5
n=32
n=9
n=20
n=20
n=21
n=21
n=22