Thrombosis Research 201 (2021) 100–112

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

# Recombinant ADAMTS13 reduces abnormally up-regulated von Willebrand factor in plasma from patients with severe COVID-19

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ARTICLE INFO

*Keywords:*
COVID-19
SARS-COV-2
von Willebrand factor
ADAMTS13
Thrombosis
Endothelium
Inflammation
rADAMTS13

ABSTRACT

Thrombosis affecting the pulmonary and systemic vasculature is common during severe COVID-19 and causes adverse outcomes. Although thrombosis likely results from inflammatory activation of vascular cells, the mediators of thrombosis remain unconfirmed. In a cross-sectional cohort of 36 severe COVID-19 patients, we show that markedly increased plasma von Willebrand factor (VWF) levels were accompanied by a partial reduction in the VWF regulatory protease ADAMTS13. In all patients we find this VWF/ADAMTS13 imbalance to be associated with persistence of ultra-high-molecular-weight (UHMW) VWF multimers that are highly thrombogenic in some disease settings. Incubation of plasma samples from patients with severe COVID-19 with recombinant ADAMTS13 (rADAMTS13) substantially reduced the abnormally high VWF activity, reduced overall multimer size and depleted UHMW VWF multimers in a time and concentration dependent manner. Our data implicate disruption of normal VWF/ADAMTS13 homeostasis in the pathogenesis of severe COVID-19 and indicate that this can be reversed *ex vivo* by correction of low plasma ADAMTS13 levels. These findings suggest a potential therapeutic role for rADAMTS13 in helping restore haemostatic balance in COVID-19 patients.

## 1. Introduction

Early experience from the COVID-19 pandemic has shown that in susceptible individuals, SARS-CoV-2 infection results in a thrombotic coagulopathy that is a frequent cause of adverse clinical outcomes [1,2]. It is proposed that coagulopathy is primarily the consequence of vascular endothelial dysfunction driven by an exaggerated host inflammatory response to SARS-CoV-2 or from the direct effect of viral replication within endothelial cells [2,3]. In severe COVID-19, this results in the deposition of platelet and fibrin rich thrombi in small pulmonary blood vessels [4] and sometimes thrombosis in large peripheral veins or arteries [5,6].

In line with this model of pathogenesis, plasma markers of endothelial activation are consistently elevated in severe COVID-19 and correlate with adverse outcomes [2]. These include von Willebrand factor (VWF), which is a critical mediator of adhesive interactions between circulating platelets and the damaged vessel wall in the normal haemostatic response [7]. VWF is synthesised by endothelial cells, stored in Weibel-Palade bodies and released as ultra-high molecular weight (UHMW) multimers. These UHMW multimers are potent mediators of platelet adhesion to other platelets and to subendothelial structures exposed upon vessel damage. However, under normal

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https://doi.org/10.1016/j.thromres.2021.02.012
Received 20 November 2020; Received in revised form 14 January 2021; Accepted 8 February 2021
Available online 18 February 2021
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circumstances UHMW VWF multimers are rapidly cleaved to smaller sized multimers with lower thrombogenic potential by the plasma protease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif member 13) [8]. Disorders such as thrombotic thrombocytopenic purpura (TTP) in which there is severe autoimmune-mediated or hereditary deficiency of ADAMTS13 activity result in increased levels of VWF and in particular, the persistence of circulating UHMW VWF multimers. This manifests as the formation of abnormal platelet rich thrombi in small arterioles that frequently leads to life-threatening microvascular occlusive crises and ischaemic tissue injury [9].

Since there are some similarities between the micro-occlusive vasculopathy of disorders such as TTP and the manifestations of SARS-CoV-2 infection, it has been proposed that abnormal interactions between platelets and endothelium mediated by VWF may also contribute to thrombosis in severe COVID-19 [10–12]. Recent case reports and investigations in small patient cohorts substantiate that severe COVID-19 is associated with a marked elevation in circulating VWF levels, in most reports accompanied by reduced ADAMTS13 [12–15]. The extent of reduction of ADAMTS-13 levels in COVID-19 patients may also be predictive of adverse outcomes [16]. In order to further investigate the effect of SARS-CoV-2 infection on the VWF-ADAMTS13 axis, we performed a detailed analysis of VWF and ADAMTS13 in plasma from patients with severe COVID-19. We also studied the effects of an rADAMTS13 drug candidate (TAK-755) on VWF activity and multimeric structure in patient plasma samples by *ex vivo* substitution experiments.

## 2. Methods

### 2.1. Patient selection

Patients were eligible for the investigation if they were adults ≥18 years admitted to the intensive care units (ICUs) at either University Hospitals Bristol and Weston NHS Foundation Trust ($n = 24$) or University Hospital Southampton NHS Foundation Trust ($n = 12$) with COVID-19 requiring mechanical ventilation because of severe respiratory failure. Where possible, SARS-CoV-2 infection was confirmed by PCR testing or by detection of seroconversion using specific antibody tests.

Plasma samples were identified by pathology staff by selecting approximately every third new blood sample submitted for standard care coagulation tests from the COVID-19 ICUs between 19th March and 7th May 2020 which spanned the first COVID-19 wave in the United Kingdom. Clinical data were retrieved retrospectively from electronic ICU records and then de-identified. Ethnicity data were classified according to standardized terms used in the 2001 UK Census [17]. The presence of Acute Respiratory Distress Syndrome was assessed using Berlin criteria [18]. Evidence of additional bacterial sepsis was detected from inspection of contemporaneous clinical case records and pathology records such as blood culture or bacterial sepsis biomarker results. Analyses were performed on de-identified spare plasma that remained after performing standard care diagnostic tests. Sample analysis and the linkage to clinical data were registered as service evaluation projects and were exempted from research ethics committee approval and patient consent by the host organisations in accordance with UK NHS Health Research Authority guidance [19].

### 2.2. SARS-CoV-2 detection

SARS-CoV-2 RNA was detected from nasopharyngeal swabs by PCR using the Aptima SARS-CoV-2 Assay on a Hologic Panther System (Hologic, Marlborough, MA, USA). SARS-CoV-2 seroconversion was detected using a novel serological assay reported recently [20]. The test system consists of two independent ELISAs for which antibody titres of >5 U/mL in both are required to indicate seroconversion. Specific SARS-CoV-2 patient antibodies are bound in the TECHNOZYM anti SARS CoV 2 RBD IgG ELISA (Technoclone, Vienna, Austria) using immobilized recombinant SARS-CoV-2 RBD and in the TECHNOZYM anti SARS CoV 2 NP IgG ELISA (Technoclone) using immobilized recombinant nuclear SARS-CoV-2 protein. In both ELISAs, the captured patient antibodies are detected using an anti-human IgG HRP labelled polyclonal antibody and quantified using SARS-CoV-2 positive serum standardized against a specific monoclonal antibody for RBD (CR3022).

### 2.3. Coagulation protein analyses

Blood samples collected into 3.2% Trisodium citrate were centrifuged at 2400*g* for 10 min to yield plasma aliquots which were stored at −80 °C or in temperature monitored dry ice until analysis. The prothrombin time, activated partial thromboplastin time, Clauss fibrinogen and D dimer were measured using a CS-2500 series analyzer (Sysmex Corp. Kobe, Japan) using Siemens Healthcare reagents (Marburg, Germany). The Factor VIII activity was determined using a one-stage activity assay with Actin FS activator and FVIII deficient plasma from Siemens Healthcare.

### 2.4. Von Willebrand factor assays

VWF:Ag was determined from eight different dilutions of patient plasma using the Asserachrom VWF:Ag test kit (Diagnostica Stago, Asnières sur Seine, France) with human normal plasma as the assay reference standard. VWF ristocetin cofactor activity (VWF:RCo) was measured using the BCS coagulation system analyzer (Behring Coagulation System, BCS, Siemens, Germany) and a reference plasma, calibrated against the WHO standard. VWF collagen binding (VWF:CBA) was determined using the TECHNOZYM VWF:CBA test kit (Technoclone, Vienna, Austria) or using the Zymutest test kit (HYPHEN BioMed SAS, Neuville sur Oise, France) using assay calibrators traceable to the WHO standard.

### 2.5. ADAMTS13 assays

Three complementary assays were used to determine ADAMTS13 levels, all obtained from Technoclone (Vienna, Austria). TECHNOZYM ADAMTS13 Activity ELISA is a chromogenic assay in which the endpoint is proteolysis of the substrate GST-VWF73 [21,22]. TECHNOFLUOR ADAMTS13 Activity is an automated assay for the Ceveron s100 instrument which uses a FRET substrate based on the VWF73 peptide [23]. TECHNOZYM ADAMTS13 Antigen ELISA is a sandwich ELISA employing anti-ADAMTS 13 antigen directed to the CUB domain and secondary HRP labelled anti-ADAMTS13 polyclonal antibodies [24]. All ADAMTS13 parameters were calculated using assay specific reference plasma calibrated against the WHO standard. The COVID-19 patient samples were analysed alongside a control plasma sample from a 48 year old male with autoimmune TTP (known ADAMTS13 activity <0.01 IU/mL) and a normal pooled plasma sample (ADAMTS13 activity 0.9–1.0 IU/mL; Precision BioLogic, Dartmouth, NS, Canada).

### 2.6. VWF multimer composition

Analysis of VWF multimeric composition was initially performed using home cast low-resolution 1% and high-resolution 2.5% SDS agarose gels (SeaKem, Lonza, Basel, Switzerland) which are optimized to separate VWF multimers >20 MDa which are larger than those present in normal human plasma. In order to minimise infectivity before open electrophoresis using the home cast gels, the plasma samples underwent heat inactivation for 1 h at 60 °C. The samples were then diluted so that 1 IU VWF:Ag/mL was applied to each electrophoresis gel lane to assist direct visual comparison between lanes and quantitative densitometric scanning. Multimer bands were detected by in-gel 2-step immunostaining with an anti-VWF antibody (DAKO, Glostrup; Denmark) and a goat-anti-rabbit ALP conjugate (Jackson, West Grove, PA, USA).

Multimer bands were visualised with an AP conjugate substrate kit (Bio Rad, CA, USA) [25].

Multimer patterns were evaluated visually and quantified by densitometry (GS-900, BioRad, CA, USA). For each lane, the distance between the top of the separation gel and the lowest multimer band (corresponding to VWF dimers) was assigned a migration value of 1.0. The distance of the largest VWF multimer band in the sample lane was then measured and expressed as a fraction of this and denoted *relative migration distance* ($R_f$). The proportion of the total migration distance of the VWF dimer band that is occupied by all of the other multimers in the sample lane is therefore 1-$R_f$. In order to enable comparison between different electrophoresis gel runs this value was reported as a percentage of the 1-$R_f$ value for a normal plasma sample separated on the same gel (termed hereafter the *UHMW multimer quantitation parameter*). An increased percentage value indicates UHMW VWF multimers in the test sample.

The findings from the home cast gel electrophoresis were validated using a semi-automated electrophoresis gel method using the HYDRAGEL von WILLEBRAND MULTIMERS kit and a HYDRASYS 2 SCAN instrument (Sebia, Lisses, France). This method separates VWF multimers in the high to ultra-large range less well than the home cast 1% agarose gels but shows better reproducibility between gels.

### 2.7. rADAMTS13

Manufacture and characterisation of purified recombinant human ADAMTS13 (rADAMTS13, Takeda, Vienna, Austria) from a Chinese hamster ovary cell line has been described previously [26–28]. The rADAMTS13 product used in our investigation is the same as the investigational drug candidate identified as TAK-755, SHP655, or BAX 930 in clinical trials for hereditary thrombotic thrombocytopenic purpura (ClinicalTrials.gov Identifier: NCT02216084) and for sickle cell disease (ClinicalTrials.gov Identifier: NCT03997760).

### 2.8. VWF cleavage studies

Degradation of VWF with rADAMTS13 was performed under conditions where VWF is partially denatured to make the cleavage motif accessible for proteolysis. The rADAMTS13 was pre-diluted to 10 and 100 IU/mL FRETS-VWF73 activity (final concentrations between 0.5 and 10 IU/mL) and activated with $BaCl_2$ in the presence of 5 mM Tris and 1.5 M urea, pH 8.0, at 37 °C for 30 min. Activated rADAMTS13 was mixed 1 + 9 with patient plasma samples and further incubated at 37 °C for 2 and 5 h before proteolysis was stopped by the addition of $Na_2SO_4$ (8.25 mM final concentration). In control experiments to measure the effect of the endogenous ADAMTS13, the patient samples were mixed with buffer instead of rADAMTS13. Samples were centrifuged at 2500 ×*g* for 5 min and the VWF in the supernatants was analysed using the VWF:CB assay and by quantitative densitometry of semi-automated electrophoresis gels performed with the Sebia Phoresis rel. 9.2.0 software to calculate relative increase of VWF dimer levels relative to the total quantity of VWF determined from the areas under the densitometry curves. UHMW VWF multimers were quantified from the home case gels as described above.

### 2.9. Statistical analysis

Descriptive statistics and Pearson correlation analysis were performed to determine correlations (r) between ADAMTS13 and VWF levels measured with the different test methods. In order to investigate the extent of degradation of VWF by rADAMTS13 in the *ex vivo* VWF cleavage experiments, paired *t*-tests were used to compare VWF:CB assay data (10 samples) and relative VWF dimer content from semi-automated electrophoresis gels (8 samples) at baseline and after 5 h of incubation with 1 IU/mL rADAMTS13. All the statistical analyses were performed using Minitab version 18.1.

**Table 1**
Clinical and core laboratory characteristics of the study group.

| | | |
|---|---|---|
| Age (years) | 61 (23–76) | |
| Male sex | 28 (78%) | |
| Ethnicity | | |
| White British | 19 (53%) | |
| Black or Black British | 10 (28%) | |
| Asian- Pakistani or British Pakistani | 3 (8%) | |
| Other | 4 (11%) | |
| Co-morbidities | | |
| Diabetes | 15 (42%) | |
| Hypertension | 14 (39%) | |
| Obesity | 9 (25%) | |
| Cardiovascular disease | 9 (25%) | |
| Chronic kidney disease | 8 (22%) | |
| Immunocompromised | 3 (8%) | |
| Active malignancy | 3 (8%) | |
| Acute Respiratory Distress Syndrome[a] | 36 (100%) | |
| Additional bacterial infection | 16 (44%) | |
| Thrombosis | | |
| Peripheral vein thrombosis | 8 (22%) | |
| Pulmonary embolus or thrombosis | 7 (19%) | |
| Stroke | 3 (8%) | |
| No confirmed thrombosis | 19 (53%) | |
| Anticoagulation at time of blood sample | | |
| Prophylactic LMWH or UFH | 22 (61%) | |
| Treatment LMWH, UFH or Argatroban | 12 (33%) | |
| No anticoagulant | 2 (6%) | |
| Outcome | | |
| Death | 19 (53%) | |
| Discharged from intensive care | 17 (47%) | |
| ABO blood group O | 13 (36%) | Expected 48%[b] |
| ABO blood group A, B or AB | 23 (64%) | Expected 52%[b] |
| PT (s) | 11.8 (10.9–37.7) | RI 9.5–12.0 |
| APTT (s) | 28.8 (20.5–71.8) | RI. 23.0–32.0 |
| Clauss fibrinogen (g/L) | 6.4 (3.2–9.0) | RI. 1.5–4.5 |
| Platelet count ($\times 10^9$/L) | 231 (11–621) | RI. 150–400 |
| D-dimer (ng/mL) | 3128 (235–13,280) | RI. <500 |
| Factor VIII activity (IU/mL) | 4.71 (2.14–9.98) | RI. 0.50–2.00 |

The clinical data are number (%). The laboratory data are median (range) reported alongside reference intervals (RI) derived from analysis of plasma from healthy volunteers. LMWH- low molecular weight heparin; UFH- unfractionated heparin; PT- prothrombin time; APTT- activated partial thromboplastin time

[a] Classified according to Berlin ARDS criteria [18].

[b] UK Blood donor registry expected frequencies.

## 3. Results

### 3.1. Characteristics of the cases

All 36 patients enrolled into the investigation displayed a rapidly progressive febrile illness with cough, shortness of breath, pulmonary infiltrates and severe respiratory failure requiring mechanical ventilation that are characteristic features of severe COVID-19. Thirty-four patients had a positive SARS-CoV-2 PCR test of which 30 had developed specific SARS-CoV-2 antibodies. One patient had a negative PCR test but displayed high levels of SARS-CoV-2 antibodies indicating infection. A positive detection test was not obtained from a single patient with who died from rapidly progressive COVID-19 before PCR confirmation could be performed. The patients spent a median of 16 days on the ICU before discharge to a low dependency ward or death. Blood samples for laboratory analysis were collected a median of 10 days (range 0–57 days) after ICU admission. All except two patients were receiving either prophylactic or therapeutic doses of parenteral anticoagulants at the time of blood sampling. All of the patients displayed greatly elevated plasma fibrinogen, D-dimer levels and Factor VIII activity consistent with severe activation of the hemostasis pathway. No patients had the laboratory features of disseminated intravascular coagulation according to ISTH criteria [29]. The clinical and core laboratory characteristics of the

**A.**

| Laboratory parameter | Patient value (n=36) | | Reference range |
|---|---|---|---|
| | Median | Range | |
| VWF antigen (IU/mL) | 4.45 | 1.22 - 10.32 | 0.50 - 1.60 |
| VWF ristocetin co-factor activity (IU/mL) | 3.27 | 1.28 - 9.20 | 0.58 - 1.72 |
| VWF collagen binding activity (IU/mL) | 4.38 | 1.80 - 9.38 | 0.40- 2.50 |
| ADAMTS13 activity ELISA (IU/mL) | 0.42 | 0.21 - 0.84 | 0.66 - 1.10 |
| ADAMTS13 activity FRETS (IU/mL) | 0.52 | 0.21- 0.89 | 0.60 - 1.21 |
| ADAMTS13 antigen (IU/mL) | 0.38 | 0.08 - 0.73 | 0.41 - 1.41 |
| UHMW VWF multimers present by visual examination and densitometry | 36 (100%) | n.a. | n.a*. |
| UHMW multimer quantitation (%) | 115 | 107 - 122 | n.a.* |

**B.**

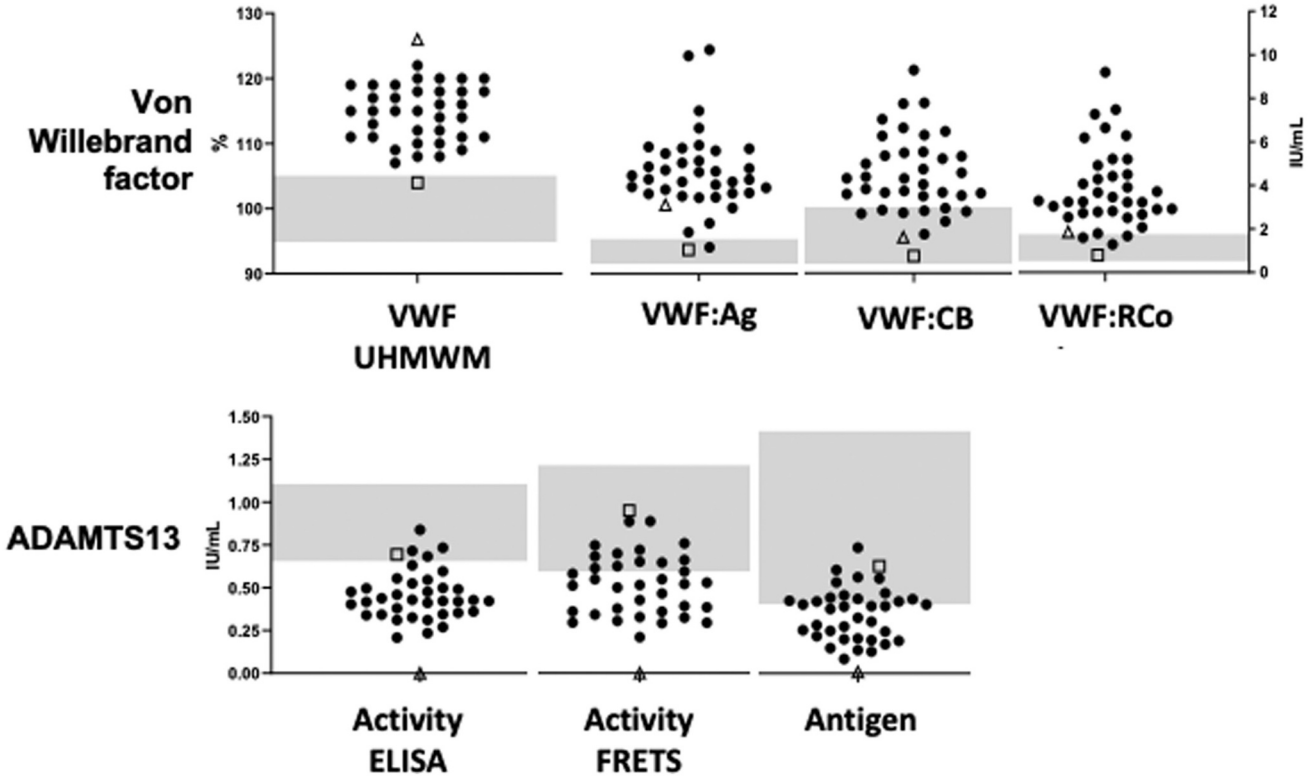
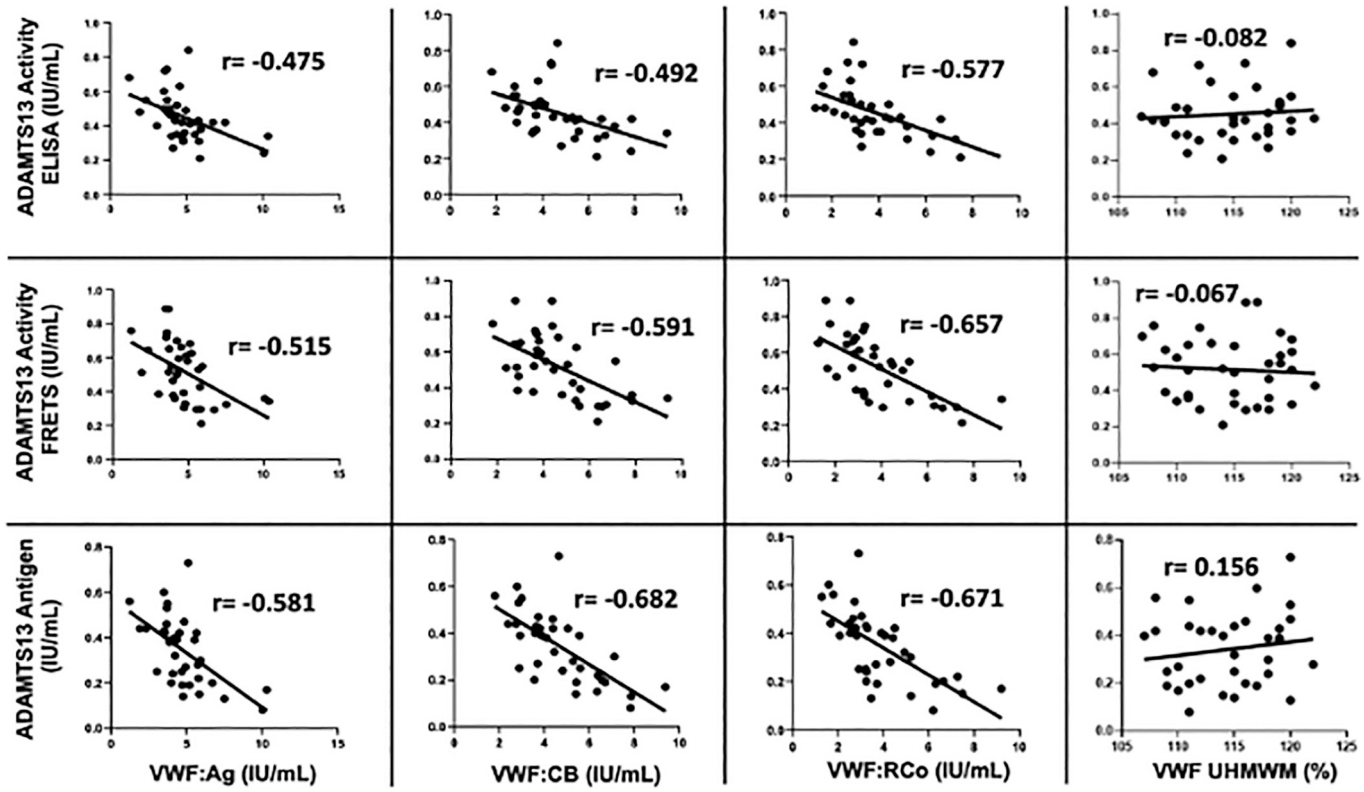



**Fig. 1.** VWF and ADAMTS13 levels in 36 patients with severe COVID-19.
A: Summary statistics presented as median and range alongside reference intervals obtained from analysis of healthy volunteer samples. The UHMW VWF multimer quantitation parameter is the proportion of the migration distance of the VWF dimers that was occupied by all VWF multimers in each sample lane expressed as a percentage value of that in the healthy control plasma lane from the same gel.
VWF - von Willebrand factor; UHMW - ultra high molecular weight; ADAMTS13 - a disintegrin and metalloprotease with a thrombospondin type 1 motif member 13; n.a.- not applicable. * normal human plasma is devoid of UHMW VWF multimers. B: Assay results from all patient samples and controls. COVID-19 patient samples (circles); Acute phase TTP control (triangle); Normal Plasma control (squares); the grey boxes indicate the reference intervals.

**A.**




**B.**

| Parameter | Pearson r | p-value | Correlation |
|---|---|---|---|
| ADAMTS13 activity ELISA / VWF:Ag | -0.475 | 0.003 | moderate |
| ADAMTS13 activity FRETS / VWF:Ag | -0.515 | 0.001 | moderate |
| ADAMTS13 antigen / VWF:Ag | -0.581 | < 0.001 | moderate |
| ADAMTS13 activity ELISA / VWF:CB | -0.492 | 0.002 | moderate |
| ADAMTS13 activity FRETS / VWF:CB | -0.591 | < 0.001 | moderate |
| ADAMTS13 antigen / VWF:CB | -0.682 | < 0.001 | strong |
| ADAMTS13 activity ELISA / VWF:RCo | -0.577 | <0.001 | moderate |
| ADAMTS13 activity FRETS / VWF:RCo | -0.657 | <0.001 | strong |
| ADAMTS13 antigen / VWF:RCo | -0.671 | <0.001 | strong |
| ADAMTS13 activity ELISA / % VWF UHMWM | -0.082 | 0.634 | no correlation |
| ADAMTS13 activity FRETS / % VWF UHMWM | -0.067 | 0.699 | no correlation |
| ADAMTS13 antigen / % VWF UHMWM | 0.156 | 0.362 | no correlation |

**Fig. 2.** Relationship between von Willebrand Factor laboratory parameters and ADAMTS13 activity in the COVID-19 samples.
A: Graphical relationship between the laboratory parameters showing lines of best fit form the correlation analysis using the Pearson test. B: Correlation estimates as r-values and associated *p*-values. Correlations were classified as strong (r: 0.7 to 1.0), moderate (r: 0.5 to 0.7), weak (r: 0.3 to 0.5) or no correlation (r: 0 to 0.3).

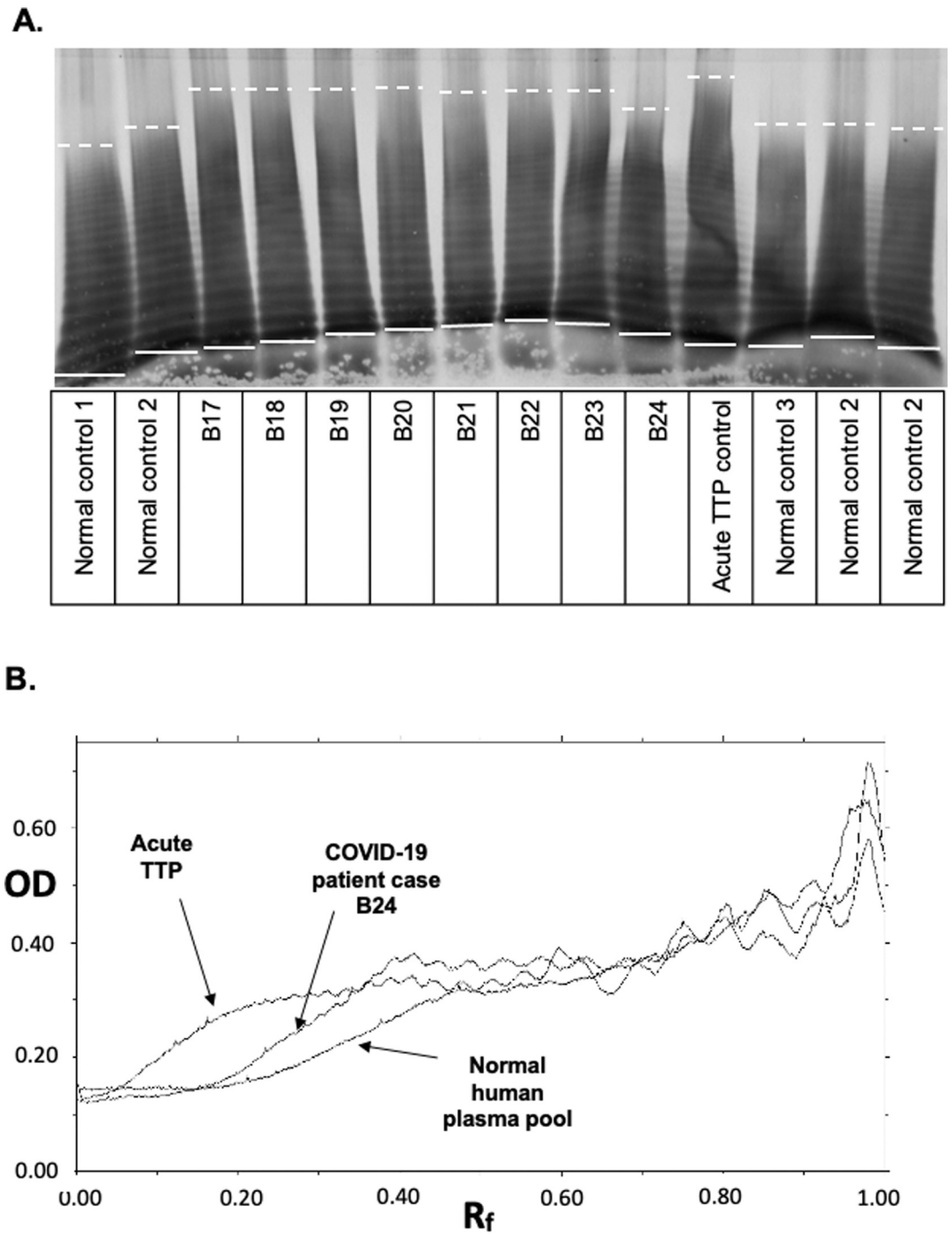



**Fig. 3.** Electrophoretic analysis of von Willebrand factor multimers from patients with severe COVID-19.
A: COVID-19 plasma samples (B17-B24) were separated by electrophoresis on a 1% agarose gel. VWF multimers were visualised after immunostaining with an anti-VWF primary antibody followed by a secondary goat-anti-rabbit ALP conjugate. Normal control 1- pooled normal plasma after virus inactivation by heat treatment; Normal control 2- pooled normal plasma without heat inactivation; Normal control 3- volunteer healthy donor travelling control plasma; Acute TTP control- sample from a patient with acute autoimmune TTP. The solid lines indicate the fastest migrating band in each lane which corresponds to VWF dimers. The broken lines indicate the upper limit of the stainable part of each lane indicating the largest VWF multimers. B: Representative densitometric scan of the sample lane for a COVID-19 patient (B24), for a patient with acute autoimmune TTP and for a pooled normal plasma sample. The x axis represents the distance in arbitrary units from the upper end of the separation gel (designated 0.0) and the lowest molecular weight band corresponding to the VWF dimers designated as 1.0. The y axis is the optical density.

patients are reported in Table 1.

### 3.2. Von Willebrand factor and ADAMTS13 levels

All of the COVID-19 patients had plasma VWF protein levels (VWF:Ag) above the upper limit of our laboratory reference interval derived from healthy volunteer plasma samples. In one third of cases, VWF levels were more than five-fold higher than average normal levels and in two cases were more than ten-fold higher (Fig. 1). There was a corresponding dramatic increase in plasma VWF activity determined using both the VWF:RCo and VWF:CB assays.

By contrast, levels of ADAMTS13 protein (ADAMTS antigen) and

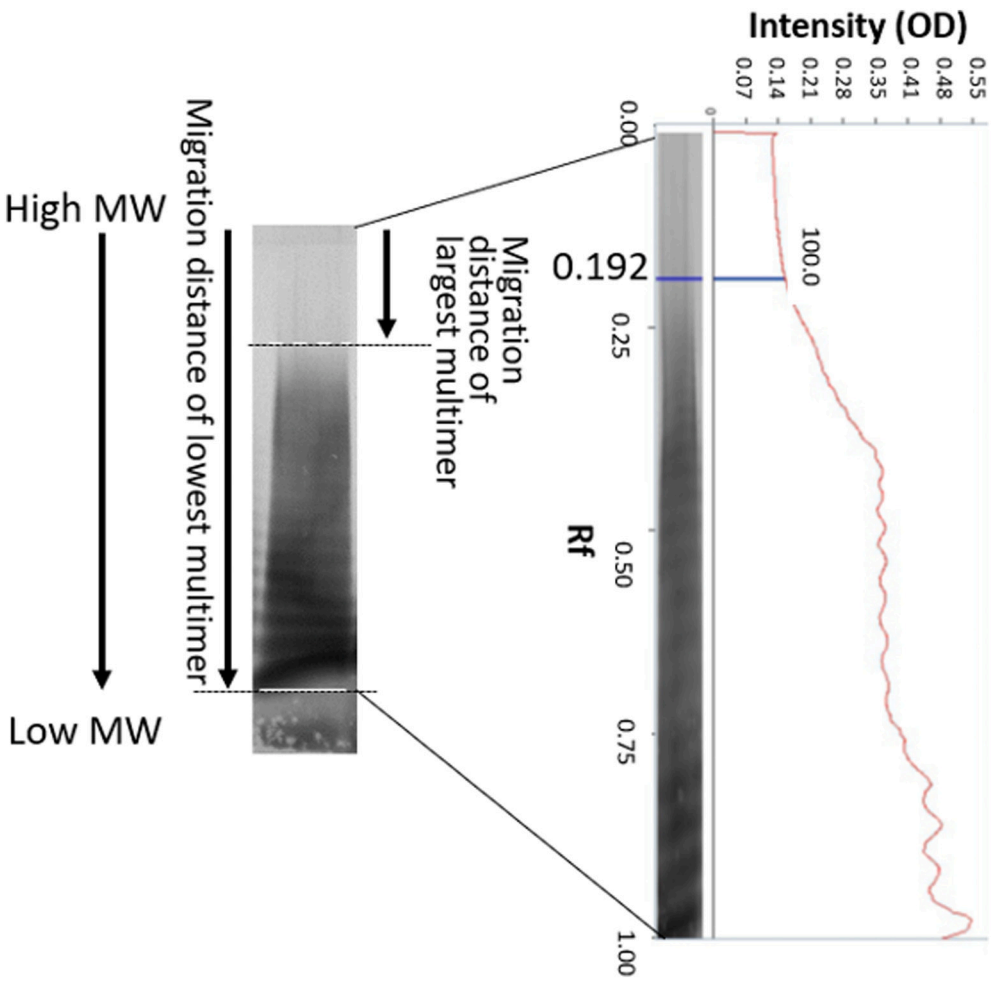



**Fig. 4.** Example electrophoretic determination of multimer size in plasma from patient B26.

The plasma sample from the patient was adjusted to 1 IU/ mL VWF:Ag and separated on a 1% agarose gel followed by immunostaining and densitometry. The distance between the top of the separation well and the lowest multimer band (VWF dimers) was assigned a migration value of 1.0. In this example the relative migration distance (Rf) of the largest VWF multimer was 0.192 that of the lowest multimer band. The proportion of the total migration distance of the VWF dimer band that is occupied by larger VWF multimers is therefore 1-Rf, in this example calculated as 1.000–0.192 = 0.808. The 1-Rf value for normal plasma separated on the same gel was 0.729 (not shown). Therefore, the proportion of the patient sample lane containing VWF multimers (UHMW multimer quantitation parameter) is 111% (0.808/0.729*100 = 111%) that of control reflecting the UHMW multimers near the top of the lane.

activity determined both by the ELISA and FRETS assays were in most patients reduced to between 30 and 70% of average normal levels (Fig. 1). No COVID-19 patients displayed ADAMTS13 activity levels less than 20% of average normal levels, distinguishing these from the autoimmune TTP control in which ADAMTS13 activity was undetectable by any of the assays. VWF levels determined using activity and antigen tests showed a strong to moderate negative correlation with ADAMTS13 levels (Fig. 2).

### 3.3. Von Willebrand factor multimer analysis

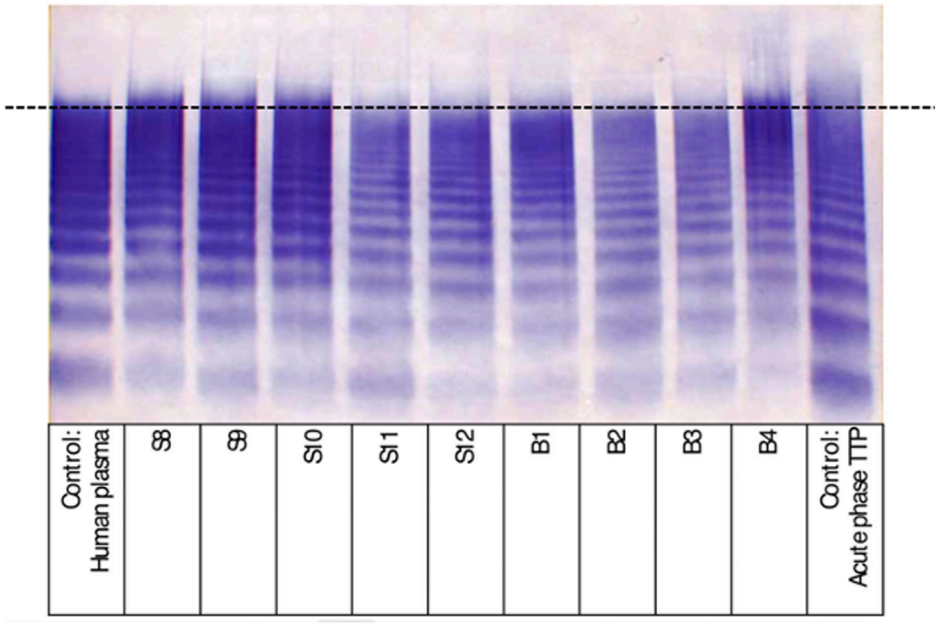
Visual inspection of the immunostained low-resolution agarose gels showed that UHMW VWF multimers were present in all 36 of the COVID-19 patients. As expected, UHMW VWF multimers were also present in the control sample from a patient with autoimmune TTP and were absent in all of the control samples from healthy volunteers (Fig. 3). The additional UHMW VWF multimers resulted in an increase in the UHMW multimer quantitation parameter to between 107% and 122% that of control plasma (Figs. 1A, 3). The highest increase in the COVID-19 samples approached that of the control with autoimmune TTP (126%). The methodology for quantitation of UHMW multimers from illustrative case B26 is also shown in Fig. 4. The presence of UHMW VWF multimers did not correlate with ADAMTS13 levels (Fig. 2A) reflecting that although quantitation of UHMW VWF forms using gel electrophoresis is robust within individual samples, comparison of values between different samples is limited by intrinsic variation in staining intensity between gel lanes.

The finding of abnormal UHMW VWF multimers was confirmed by quantitative densitometric analysis of the home cast gels and by visual inspection of the independent semi-automated electrophoresis assay (Fig. 5). The semi-automated system improves resolution of lower molecular weight VWF multimers and was used to quantify VWF dimers in the subsequent rADAMTS13 incubation experiments. This high-resolution system is less suitable to separate the UHMW VWF multimers which have a molecular weight of approximately 20 MDa. However, although unsuitable for quantitation of UHMW VWF multimers, the semi-automated gels were sufficient to enable visual confirmation of UHMW multimers in all of the COVID-19 patient samples. Illustrative examples of semi-automated electrophoresis for the COVID-19 samples and quantified UHMW multimers determined from the corresponding home-cast gels are shown in Fig. 5A and B respectively.

### 3.4. Ex vivo incubation with rADAMTS13

In order to investigate the effect of restoring the low ADAMTS13 levels on VWF in the patient samples, we incubated 10 plasma samples from COVID-19 patients with different concentrations of rADAMTS13 and monitored VWF activity and multimer composition over time. In plasma from illustrative patient S12 (VWF:Ag 4.36 IU/mL, ADAMTS13 0.59 IU/mL; VWF/ADAMTS13 ratio 7.4) there was a small reduction in VWF activity at 2 and 5 h consistent with the presence of endogenous ADAMTS13. However, after supplementation with 0.5 or 1 IU/mL rADAMTS13 predicted to restore plasma ADAMTS13 to approximately 100% (1 IU/mL) and 150% (1.5 IU/mL) of average normal levels respectively, there was an accelerated reduction in VWF activity in a dose and time dependent manner (Fig. 6A). After incubation with 1 IU/

A.




B.

| | Normal | S8 | S9 | S10 | S11 | S12 | B1 | B2 | B3 | B4 | Acute TTP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| UHMW VWF* (%) | 100 | 109 | 119 | 120 | 113 | 119 | 122 | 118 | 120 | 110 | 126 |

**Fig. 5.** VWF multimers from patients with severe COVID-19 visualised using the semi-automated electrophoresis system. A: Plasma samples of 9 patients with COVID-19 were separated by semi-automated electrophoresis using the HYDRAGEL von WILLEBRAND FACTOR MULTIMERS kit and a HYDRASYS 2 instrument. Each sample was adjusted to 1 IU VWF:Ag per mL, separated and stained for multimers in parallel. Control samples from a patient with acute TTP and a healthy volunteer was applied to the same gel. The broken line indicates the largest stainable part of the normal human plasma control. The migration distances of differently sized ultra-high molecular weight multimers were less pronounced as with the home-cast low resolution gels. Although, this prevented reproducible quantitation, the UHMW VWF multimers were clearly evident as abnormal immunostaining material above the dotted line. B: UHMW VWF quantified from the same samples from home cast gels using the analysis method presented in Fig. 4.

mL rADAMTS13 for 5 h, the mean value of VWF:CB in the samples from all 10 patients that were tested decreased from 3.01 (SD: 1.37; SEM: 0.434) to 1.64 (SD: 0.89; SEM: 0.28) IU/mL ($p < 0.001$; t-value 7.18; Fig. 6B,C and Table 2). This decrease in VWF activity was accompanied by a statistically significant increase ($P < 0.001$; t-value −7.04) in VWF dimers measured by densitometry (Table 2).

The effect of supplementing ADAMTS13 to higher levels was tested using plasma from patient B1 in which there was a greater VWF/ADAMTS13 mismatch (VWF:Ag 5.76 IU/mL; ADAMTS13 0.43 IU/mL; ratio 13.4). Incubation with 10 IU/mL rADAMTS13 resulted in a progressive reduction in VWF activity over time achieving more than a 60% reduction at 5 h (Fig. 7A) and disappearance of all the abnormal UHMW VWF multimers visualised using the semi-automated electrophoresis method (Fig. 7B). The effect of 1 IU/mL rADAMTS13, was less pronounced although this was still sufficient to cause a reduction in VWF activity and a visible depletion of UHMW VWF within 2 h. Disappearance of UHMW VWF was accompanied by marked increase in low molecular weight multimers and dimers (Fig. 7C). These findings were also present with the home cast gels which showed that the elevated UHMW multimer quantitation parameter of 124% reduced by 16% after 5 h of incubation with 1 IU/mL rADAMTS13 and by 36% with 10 IU/mL rADAMTS13, to a level lower than that observed in control plasma (88%). Exposure of VWF to rADAMTS13 resulted in the appearance of higher quantities of lower sized multimers with an increase in stainable satellite bands indicative of proteolytic cleavage of VWF.

## 4. Discussion

In this exploratory investigation, we evaluated the VWF/ADAMTS13 axis in adults admitted to two hospitals for mechanical ventilation due to severe COVID-19. The liberal inclusion criteria enabled enrolment of a cross-sectional cohort of patients early in the course of severe COVID-19 and also those with established disease requiring prolonged ventilation. The cohort contained a preponderance of older males with multiple comorbidities and was over-represented with patients from black and ethnic minority groups and with non-O blood groups. These characteristics, and the frequency of adverse outcomes mirrors previously reported severe COVID-19 cohorts [30,31].

The striking finding in this clinically heterogeneous cohort was that all patients had substantially elevated circulating VWF levels that in some cases were up to ten-fold higher than normal. We believe that the extent to which VWF is dysregulated in this cohort is greater than in any previously reported inflammatory illness and reproduces previous observations in severe COVID-19 patients [2,10,12,15]. We also show that most patients had decreased circulating levels of ADAMTS13 protein and ADAMTS13 proteolytic activity in most cases to between 30% to 70% of normal levels, resulting in a substantially abnormal VWF/ADAMTS13 ratio. These data confirm other recent observations from

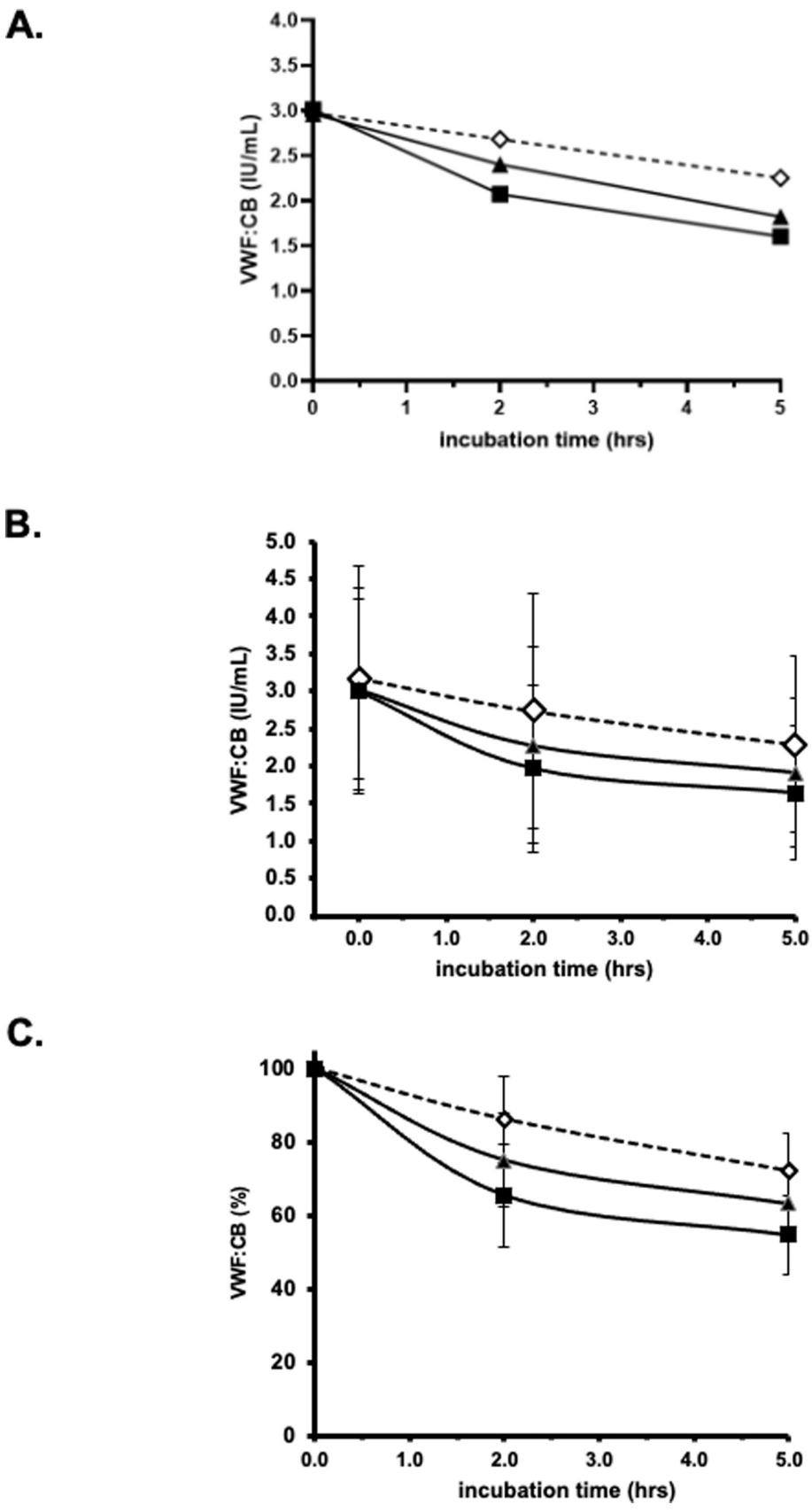



**Fig. 6.** Incubation of plasma samples with 0.5 or 1.0 IU/mL rADAMTS13.
A: Plasma from illustrative severe COVID-19 patient S12 (VWF/ADAMTS13 ratio of 7.4) was incubated with 0.5 IU/mL (triangles) or 1 IU/mL (squares) rADAMTS13 or without ADAMTS13 (diamonds). Sub-samples were taken immediately after addition of rADAMTS13, and at 2 and 5 h and analysed for VWF activity by the collagen binding assay. B: Pooled absolute VWF:CB results from plasma samples from 10 severe COVID-19 patients labelled as in graph A. C: Pooled VWF:CBA values labelled as in graph A but with values expressed as a percentage of the value at 0 h. For both of the pooled data, the points are means and the error bars are standard deviations. There was a significant decrease in VWF:CBA ($P < 0.001$; t-value −7.04) after incubation for 5 h compared with baseline values.

**Table 2**
VWF cleavage in plasma samples from 10 COVID-19 patients following *ex vivo* incubation 1 IU/mL rADAMTS13.

| Sample number | VWF:CB (IU/mL) | | Percentage decrease in VWF:CB (0 *vs.* 5 h) | VWF dimer relative to total VWF multimers (AUC %) | | Percentage increase in VWF dimer (0 *vs.* 5 h) |
|---|---|---|---|---|---|---|
| | 0 h | 5 h | | 0 h | 5 h | |
| S21 | 3.70 | 1.63 | 44 | 4.9 | 8.5 | 173 |
| S6 | 2.19 | 0.73 | 33 | 5.4 | 7.0 | 130 |
| S10 | 6.48 | 3.91 | 60 | n.d. | n.d. | – |
| S12 | 3.01 | 1.60 | 53 | n.d. | n.d. | – |
| B1 | 3.09 | 2.02 | 65 | 3.6 | 9.1 | 253 |
| B2 | 1.39 | 0.84 | 60 | 5.4 | 11.3 | 209 |
| B5 | 2.46 | 1.61 | 65 | 3.7 | 11.0 | 297 |
| B7 | 3.03 | 1.58 | 52 | 5.3 | 10.1 | 191 |
| B15 | 2.44 | 1.08 | 44 | 5.1 | 12.3 | 241 |
| B18 | 2.25 | 1.42 | 63 | 3.6 | 7.0 | 194 |

n.d. not done;

COVID-19 case series of reduced ADAMTS13 activity [10,13–15] or markedly increased VWF/ADAMTS13 ratio [12,32,33]. Although in a further small case series in which comprehensive hemostasis testing was performed longitudinally in COVID-19 patients for the duration of hospital admission, reduced ADAMTS13 was an inconsistent finding [34]. It is noteworthy that low ADAMTS13 plasma levels predicted mortality in COVID-19 patients in three independent studies [16,35,36].

One possible explanation for reduced ADMATS13 levels is sequestration of ADAMTS13 by high levels of circulating high molecular weight VWF multimers through a low affinity interaction that does not result in multimer cleavage [37]. Low ADAMTS13 levels may also be a consequence of the cytokine storm that accompanies severe COVID-19 because normal ADAMTS13 synthesis from cultured human cell lines is inhibited by inflammatory cytokines such as IFN-γ, IL-4 and TNF-α [38]. Inflammatory cytokines also inhibit ADAMTS13-mediated cleavage of UHMW VWF multimers [39].

We also show that in all of the COVID-19 patients, these reciprocal changes in the plasma levels of VWF and ADAMTS13 were accompanied by abnormal circulating UHMW VWF multimers that are completely absent in healthy controls. Circulating UHMW VWF multimers have been observed previously with trauma [40], sepsis-associated disseminated intravascular coagulation [41], severe malaria [42] and after endotoxin infusion in healthy volunteers [43] most likely because of an excess of the VWF substrate compared to ADAMTS13 resulting in incomplete VWF cleavage. Although approaching half of our patients had presumptive evidence of bacterial infection, particularly localised to intravascular lines, none displayed DIC or any other previously recognised cause for VWF/ADAMTS13 mismatch. The extent of mismatch was also much greater than reported in other inflammatory settings, resulting in dramatically higher levels of potentially thrombogenic UHMW VWF multimers.

These observations directly support the recently proposed hypothesis that coagulopathy following SARS-CoV-2 infection is driven by a potent inflammatory endotheliopathy resulting in disordered regulation of multiple components of hemostasis, including the coagulation and fibrinolysis pathways as well as the platelet-endothelium interaction [44,45]. Our findings specifically implicate abnormal release of granular stores of VWF and dysregulated proteolysis of VWF in the pathogenesis of thrombosis as has been proposed by others [3,39,46]. However, to our knowledge our investigation is the first to directly visualise that a consequence of VWF/ADAMTS13 mismatch is the presence of UHMW VWF multimers in COVID-19 plasma samples. The UHMW VWF multimers levels in some COVID19 patients were approaching those of the acute TTP control. However, even though micro-vascular occlusion is a feature of both COVID-19 and acute TTP, the COVID19 coagulopathy is distinct because of the absence of TTP features such as severe thrombocytopenia, circulating red cell schistocytes and severe reduction in ADAMTS13 level. Therefore, our findings fit best with a model in which VWF dysregulation is an important component of COVID-19 coagulopathy but that dysfunction of other haemostatic pathways are contributory [44].

Our findings are potentially significant for the management of patients with COVID-19 because the VWF-ADAMTS13 axis is a therapeutically accessible target. We show early evidence of the potential utility of ADAMTS13 replacement by showing that incubation of patient samples with rADAMTS13 resulted in a time and concentration dependent reduction in VWF activity, even in the sample with the most severe mismatch between VWF and ADAMTS13. We showed further that this correction of abnormally high VWF activity was accompanied by the disappearance of UHMW VWF multimers and a time-dependent increase in lower molecular weight multimers with more intense satellite bands indicating *ex vivo* proteolysis. This pharmacodynamic effect of ADAMTS13 replacement mirrors that observed in other coagulopathies in which rADAMTS13 has been evaluated in early phase clinical trials including in patients with VWF/ADAMTS13 mismatch because of severe congenital TTP [28] and in plasma samples from patients with autoimmune TTP [47]. rADAMTS13 also results in dissolution of thrombus in flow chamber models [48] and in a small animal model [49] of VWF/ADAMTS13 mismatch. In our study, *ex vivo* proteolytic activity in the COVID-19 plasma samples was even observed after correction of ADAMTS13 levels to 1.0 to 1.5 IU/mL, which was in the range achieved in the phase 1 congenital TTP study following infusion of 40 IU/Kg rADAMTS13 which was well tolerated and was not associated with significant adverse events [28]. However, it is also noteworthy that the ADAMTS13 activity required to cleave abnormal UHMW VWF multimers in static models may be lower than in conditions of flow [50]. Therefore, caution should be used in extrapolating the effective rADAMTS13 concentrations observed in our study to the clinical setting. We also highlight a potential clinical caution of bleeding associated with the small reduction in high molecular weight VWF multimers as well as the UHMW forms observed in our *ex vivo* experiments.

Although anticoagulation was associated with lower mortality among hospitalized COVID-19 patients [51] it is significant that antithrombotic drugs such as heparin that target the coagulation pathway may be less effective in preventing thrombosis in severe COVID-19 than in other inflammatory diseases [52]. The dramatic abnormalities in the VWF-ADAMTS13 axis demonstrated in our investigation provide a potential explanation for this. Our demonstration that ADAMTS13 replacement restores VWF homeostasis in COVID-19 plasma samples suggests that a purified rADAMTS13 should be considered as a potential therapeutic intervention for COVID-19 coagulopathy alongside other anti-thrombotic therapies.

## Authorship contributions

PLT and AM designed the experiments and wrote the manuscript. GS, HG, and NBB performed and interpreted the experiments. ML, SR, RP, CRS, RK, RD, RK, AD and IJ reviewed patient data and the manuscript. BE, BM, WE, and NJ reviewed the experimental data and the manuscript.

## Declaration of competing interest

PLT, GS, HG, BE, BM, WE, and NJ are employees of subsidiaries of Takeda pharmaceutical company limited and are Takeda stock owners. ML and SR have received funding support from Takeda. NBB is an employee of Technoclone. AM, RP, CRS, RD, RK and AD declare no relevant conflicts of interest.

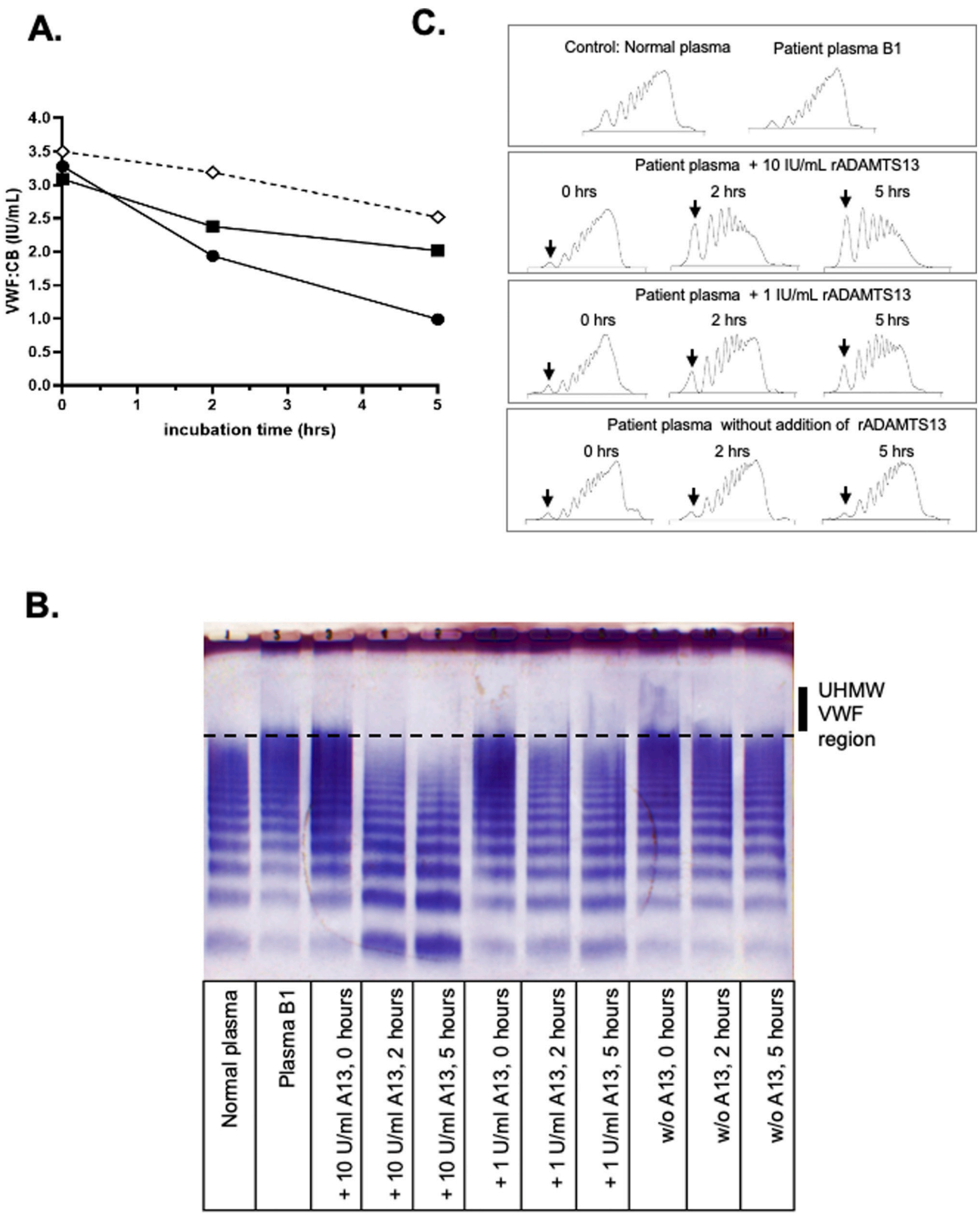



**Fig. 7.** Incubation of plasma sample B1 with 1.0 or 10.0 IU/mL rADAMTS13.
Plasma from severe COVID-19 patient B1 (VWF/ADAMTS13 ratio of 13.4) was incubated with 1.0 IU/mL (triangles) or 10.0 IU/mL (squares) rADAMTS13 or without ADAMTS13 (diamonds). Samples were taken immediately after addition of rADAMTS13, and at 2 and 5 h. A: VWF activity by the collagen binding assay; B: multimer composition visualised using the semi-automated electrophoresis gel system (Sebia) C: The corresponding gel densitograms with the VWF dimer peaks arrowed and high molecular weight VWF multimers to the right of each trace. The vertical axis represents the intensity of gel staining in arbitrary units. The control samples were normal human plasma, B1 plasma without the addition of any reagents and no incubation, and B1 plasma incubated under identical conditions but without the addition of rADAMTS13.

## Acknowledgements

We thank Margaret Griffiths, Nina Weinhappl, Margit Stimpfl, Manfred Billwein, Sylvia Peyrer-Heimstaett, Michaela Schaedler and Jutta Schreiner for expert technical support with assays and VWF electrophoresis. We are grateful to Marietta Turecek for suggesting investigation of the role of ADAMTS13 in COVID19. AM and RP are supported by the NIHR Bristol Biomedical Research Centre at University Hospitals Bristol and Weston NHS Foundation Trust and the University of Bristol. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

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