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COVID-19 and biomarkers of thrombosis: focus on von Willebrand factor and extracellular vesicles

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Abstract
COVID-19, caused by the SARS-CoV-2 virus, is responsible for a pandemic of unparalleled portion over the past century. While the acute phase of infection causes significant morbidity and mortality, post-acute sequelae that can affect essentially any organ system is rapidly taking on an equally large part of the overall impact on human health, quality of life, attempts to return to normalcy and the global economy. Herein, we summarize the potential role of von Willebrand Factor and extracellular vesicles toward understanding the pathophysiology, clinical presentation, duration of illness, diagnostic approach and management of COVID-19 and its sequelae.

Keywords Thrombosis · Biomarkers · COVID-19 · von Willebrand factor

Highlights

- COVID-19 has both acute and long-term clinical sequelae. Post-acute sequelae of SARS-CoV-2 infection or PASC is responsible for poor quality of life, impaired productivity, high health care costs and a serious economic impact. An ability to understand the pathophysiology, management and predictors of outcomes is of paramount importance.

Introduction

Circulating biomarkers may be useful in the diagnosis and management of patients with COVID-19 (reviewed in Grobler) [1]. Levels of vascular and thrombosis-related biomarkers, including those reflecting thrombin generation, fibrin formation, fibrin degradation, platelet activation and extracellular trap formation for example may offer insight for the acuity and stage of disease (reviewed in Becker) [2]. The potential role of biomarkers to gauge targeted therapies and to direct optimal management will require thorough investigation. The following review highlights the potential role of von Willebrand Factor, extracellular vesicles and platelet-leukocyte aggregates in the pathophysiology of COVID-19 and potentially its management.

von Willebrand factor

General constructs

The dynamic nature of von Willebrand Factor (VWF), based on its sites of synthesis and storage in megakaryocytes, vascular endothelial cells (ECs) and platelets, reflects several concomitant events, including EC activation and injury, megakaryocyte mobilization, and platelet activation. The greater the stimulus for virus-induced cytotoxicity, proinflammatory cytokine production, cell-free nucleic acid-associated cellular injury and impaired VWF clearance mechanisms, the higher the level measured in peripheral blood (reviewed in Becker) [3]. What role might VWF play in portending clinical events, particularly thrombosis (arterial,

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venous, microvascular)? Should it be measured routinely? Might it represent target for treatment with a likelihood of improving clinical outcome?

What is von Willebrand factor?

Von Willebrand Factor (VWF) is a glycoprotein ranging in size from 600,000 to either 20 million Daltons that participates actively in platelet adhesion to injured, disrupted or activated ECs and high-shear stress associated platelet aggregation [4]. It also actively participates in both immune and inflammatory processes (described in subsequent sections).

What is its origin?

Megakaryocytes and ECs synthesize VWF. There are several distinct pathways of secretion. The *first* represents a constitutive pathway linked directly to synthesis. The *second* is a regulated pathway involving storage of mature molecules for release following stimulation by one or more mechanical, biochemical, cellular or protein mediators [5]. Weibel–Palade bodies (WPB), containing VWF are translocated to the cell surface of platelets and ECs following activation [6]. This property could be particularly important in the setting of COVID-19 [7].

Most VWF circulating in plasma originates within endothelial cell WPBs. The VWF multimer size and the length reflects the size of WPBs, typically ranging from 0.5 to 5.0 mm, that is governed by the structural status of the Golgi apparatus where the organelles form.

Structural and functional properties

Protein sequencing of VWF has identified a 22-ammino acid hydrophobic signal peptide and a large pro-peptide that is identical to VWF antigen found within plasma. There are four homologous sequences or subunits of VWF. D repeats are found within each subunit and A repeats are in the central portion of each subunit (reviewed in Wagner) [8]. The binding sites for several ligands are localized to specific repeats of the VWF polypeptide. The crystal structures of VWFA1 and A3 repeats are known as are the three-dimensional structures of specific domains that consist of a series of short, alternating, α-helical and β-pleated sheets that form a globular domain (reviewed in Ruggeri) [4].

The pre–pro-VWF molecule consists of a 22-amino acid signal peptide, a 741 amino acid pro-peptide, and a 2050 amino acid mature subunit. The pro-vWF monomer is composed of four distinct domains (A–D). C-terminal and N-terminal intermolecular disulphide bonds form VWF multimers. The largest multimers can exceed 2×10^4 kDa and have the greatest overall functional activity. In the event of an ongoing stimulus for synthesis or release, the local concentrations of UL-VWF remain high. This is important to keep in mind when considering VWF targeted treatment in COVID-19.

Regulatory mechanisms

Fluid shear stress dynamically regulates VWF by promoting aggregation of multiple VWF units, while at the same time reducing multimer size through force-dependent cleavage by ADAMTS-13 [9]. Shear stress above 60 dyne/cm^2 is typically necessary to initiate VWF structural changes [10]. The unfolding and stretching of VWF increases in high shear stress environments that cause margination of platelets and platelet micro-particles toward the vessel wall [3, 11]. VWF, like other plasma proteins has a relatively short circulating half-life, ranging from 6 to 24 h for multimers of small-to-moderate size or molecular weight [12]. The available evidence suggests that most VWF is cleared by an active regulatory mechanism involving the liver, spleen and ECs [13] with the former being determined by its co-localization with macrophages [14].

What role might VWF play in COVID-19 phenotypes?

Pulmonary microvascular thrombosis

The proclivity for thrombosis in patients with COVID-19 presents a broad array of pathophysiologic contributions for VWF; however, we believe that pulmonary microvascular thrombosis is likely the site of greatest impact [3]. Randomized clinical trials targeting VWF with pulmonary and respiratory endpoints such as oxygen support free days will be required as a "proof of concept" in early phase investigations.

VWF and inflammation

Vascular EC express procoagulant proteins, including factor VIII and VWF under inflammatory conditions (reviewed in Mazzeffi) [15]. The degree of inflammatory stress correlates with the concentrations of interleukin (IL)-8 and tumor necrosis factor (TNF)-α. Both cytokines trigger the release of UL-VWF multimers from EC in a concentration-dependent manner [16]. Vascular ECs under stress exhibit upregulation of adhesion receptors for leukocytes and platelets. Endothelial cell WPB maturation, secretion of adhesive proteins, and exocytosis increases substantially in such conditions [17]. In turn, VWF kinetics are impacted, leading to increased surface concentrations and availability for platelet, leukocyte and leukocyte-derived extracellular trap

(ET) (primarily of neutrophil [N] origin or NETs) binding to injured or altered ECs.

VWF anchors NETs to the vessel wall and to inflamed tissues

NETs "trap" and kill bacteria [18], but they can also injure host tissues. VWF is believed to be a "linker molecule" for the binding of NETs to areas of vascular and tissue injury. The interaction between DNA and VWF takes place at the A1 domain of VWF [19]. The molecular mechanisms governing DNA-VWF interactions have been summarized by Sadoval-Perez et al. [20]. They have shown that double-stranded DNA binds to a specific helix of the VWF A1 domain via three arginine moieties. Shear stress and electrostatic guidance are the primary contributors.

VWF and angiogenesis

VWF is directly involved with maintaining normal vascular integrity. UL-VWF are the most important regulators of angiogenesis. Pathological angiogenesis occurs within the lungs of patients with severe COVID-19 (Reviewed in Becker) [3].

VWF and hypoxia

VWF expression is upregulated in heart and lung endothelial cells following hypoxia [21] and associates with the presence of thrombosis. Briefly, characterization of promotor and regulatory regions of the VWF gene are known to differ in EC located within the lung, heart, brain, and kidney vasculature. Upregulation in the lungs under hypoxic conditions occurs in both the micro-and macro-vasculature. The same is observed in EC of cardiac origin. In the absence of hypoxia VWF gene expression is confined to the brain and lung EC. VWF activation in lung EC also occurs in the presence of hypoxia [22]. The presence of megakaryocytes of bone marrow and spleen origin that reside in the pulmonary vasculature is a source of proplatelet release and platelet shedding in respond to local conditions including inflammation and hypoxia. The concomitant upregulation of VWF gene expression and activation collectively leads to a highly prothrombotic environment [23].

VWF in COVID-19

Pathology and biomarker status: endothelial cell activation and injury

Microvascular thrombosis is a hallmark of severe COVID-19. Hottz et al. [24] found that platelet activation and platelet-monocyte heterotopic aggregates occurred more readily in patients with COVID-19 complicated by ARDS compared to those with lower acuity infection. They also reported that plasma procured from severe COVID-19 patients increased activation of control platelets ex-vivo. Last, platelet-monocyte interaction was strongly associated with monocyte tissue factor expression in both patients and healthy volunteers. The effect was neutralized by blocking P-selection or the platelet αIIb/β3 receptor.

Zaid et al. [25] reported a heightened platelet activation response to low concentrations of thrombin (0.05 U/ml) in patients with COVID-19 compared to patients with ARDS from non-COVID-19 related illness. Because thrombin mediated platelet activation (reviewed in Lundblad) [26] involves the GPIbα binding site with or without engagement with protease activated receptors (PARs), an association between thrombin and VWF functional dynamics is relevant in COVID-19 [27].

The experience with VWF and ADAMTS13 in COVID-19 continues to grow, suggesting their potential role in identifying patients at risk for poor clinical outcomes following hospitalization (Table 1). Fraser et al. [28] characterized several markers of vascular EC and glycocalyx activation and injury in patients requiring admission to an Intensive Care Unit (ICU). Compared to healthy age and sex-matched controls, patients with COVID-19 had higher VWF, chondroitin sulfate, and syndecan levels. The level of each EC and glycocalyx biomarker was higher when compared to patients without COVID-19 in the ICU. Employing markers of thrombosis and EC injury, the investigators developed and trained a random forester identifier (machine-based learning) and identified predictors of mortality.

A single-center study of 68 hospitalized COVID-19 patients identified elevated VWF activity and antigen levels, particularly among those requiring ICU-level care [29], compared to non-hospitalized controls. VWF antigen ($r = 0.38$; $P = 0.001$) and soluble P-selection ($r = 0.38$; $P = 0.007$), a marker of EC injury, correlated with in-hospital mortality.

Ladikou et al. reported biomarker profiles among 24 consecutive patients with COVID-19 admitted to the ICU [30]. The overall rate of venous thromboembolism and mortality was 25% and 16.7% respectively. Factor VIII and VWF levels were elevated at 279 (251–363) μ/dl and 350 (302–433) percent (median, IQR), respectively, and correlated with clinical events. VWF levels were higher in patients who did not survive their hospitalization (median 477%) compared to those who survived (335%) ($P = 0.015$).

Rauch et al. sampled 243 adults with COVID-19 at the time of hospital admission [31]. Increased VWF:Ag and decreased Factor VIII/VWF-Ag ratio were associated with increasing oxygen requirements in both a univariate and a multivariable analysis adjusted for age, sex, body mass index, hypertension and diabetes. In a series of 88

Table 1 von Willebrand factor indices and clinical outcomes in observational studies

Investigator	N	Biomarker	Primary origin	Stimulus for release	COVID-19 population	Endpoints			Strength of Evidence
						ICU	Morbidity	Death	
Fraser [27]	10	VWF_{Ag} ↑	EC	Injury/activation	Pneumonia Hypoxia	✓			+
Goshua [28]	68	VWF_{Ag} ↑	EC	Injury/activation	ICU Non-ICU			✓	+
Ladikou [29]	24	VWF_{Ag} ↑	EC	Injury/activation	ICU High acuity ward		VTE	✓	+
Rauch [30]	293	VWF_{Ag} ↑	EC	Injury/activation	Hospitalized		VTE 0_2 requirement	✓	+ +
Bazzan [31]	88	VWF_{Ag} ↑ ADAMTS13 ↓	EC EC	Injury/activation	Hospitalized			✓	+ +
Masi [32]	28	VWF_{Ag} VWF activity	EC	Injury/activation	ARDS	ND	ND	ND ND	– –
Mancini [33]	50	VWF_{Ag}↑ $VWF_{activity}$↑ VWF propeptide ↑ VWF multimers ↑ ADAMTS13 activity ↓ VWF/ADAMTS13 ratio ↑	EC EC	Injury/activation	Hospital admission	ND	ND		+ + + + + +
Pascreau [36]	70	$VWF_{activity}$ ↑ Multimers ↓ (HMW) ADAMTS13 ↓	EC	Injury/activation	Pneumonia	✓ ✓ ✓ ✓ ✓ ✓			+
Vassiliou [37]	38	VWF_{Ag} ↑	EC	Injury/activation	ICU		✓ Trend		+/–
De Cristo [38]	11	VWF_{Ag} ↑ VWFactivity ↑	EC	Injury/activation	Hospitalized		✓ Acuity		+
Fernandez [39]	49	VWF_{Ag} ↑ ADAMTS13 activity ↓	EC	Injury/activation	Pneumonia		✓ Sepsis		+
Thomas [40]	143	VWF_{Ag} ↑	EC	Injury/activation	Mild-to-severe illness		✓ Acuity	✓	+ +

+ low, + + moderate, + + + high

ICU Intensive care unit, *VWF* Von Willebrand factor, *ND* no difference, *ADAMTS13* A disintegrin and metalloproteinase with a thrombospondin-like motif member 13, *EC* endothelial cell, *ARDS* acute respiratory distress syndrome

consecutive cases of COVID-19. Bazzan et al. identified elevated levels of VWF: Ag and reduced levels of ADAMTS-13 compared to healthy controls. Patients who died had higher levels of VWF: Ag and lower levels of ADAMTS-13 than those who survived. In addition, an ADAMTS13 plasma level < 30% was associated with increased in-hospital mortality [32].

Masi et al. [33] described coagulation-related profiles in 28 consecutive patients with ARDS-17 patients had COVID-19. Factor VIII: C was elevated in each patient with values 3–4-fold above the normal range, however, the values did not differ by infectious causes of ARDS.

Mancini and colleagues measured VWF antigen (VWF: Ag), VWF ristocetin-cofactor (VWF: RCo), VWF multimers, VWF propeptide (VWFpp) and ADAMTS13 activity in 50 patients stratified according to their intensity of care: low (requiring high-flow nasal cannula oxygenation, n = 14), intermediate (requiring continuous positive airway pressure devices, n = 17), high (requiring mechanical ventilation, n = 19). Median VWF: Ag, VWF: RCo and VWFpp levels were markedly elevated in COVID-19 patients and increased with intensity of care. By contrast, the high-to-low molecular weight VWF multimer ratios progressively decreased with increasing intensity of care [34].

Doevelaar and colleagues [35] measured VWF antigen, VWF multimers and ADAMTS13 in 75 patients with COVID-19. There was a marked increase in VWF antigen levels in patients compared with healthy controls, a higher

ratio of VWF antigen to ADAMTS13, and loss of high molecular weight multimers with increasing severity of illness. A relative reduction in ADAMTS13 activity for the level of VWF antigen suggests a processing abnormality that could contribute to altered VWF multimer profiles and a heightened state of thrombosis potential. Plasma levels of ADAMTS13 inhibitors like IL-6, thrombospondin and platelet factor 4 are often elevated in patients with severe COVID-19 [36]. Other investigators and groups have drawn similar conclusions [37, 38–41].

Henry et al. [42] identified a lower ADAMTS13 activity/VWF:Ag ratio in patients with COVID-19 and acute kidney injury and those who developed infection compared to patient with neither. In a prospective study of 50 patients with COVID-19 requiring hospital admission [34], higher D-dimer, fibrinogen and VWF:Ag levels and lower ADAMTS13 activity were observed compared to patients not requiring hospitalization. Patients requiring ICU-level care had the lowest ADAMTS13 activity.

VWF and venous thrombosis

The vasculopathy described in COVID-19 produces an environment favoring UL-VWF-platelet thrombosis either in situ or in the form of emboli that lodge within the microvasculature of vital organs-most often the lungs [43]. Morici et al. [44] reported six patients with COVID-19-one with subclavian and axillary vein thrombosis and two with bleeding events. Factor VIII, VWF:Ag, VWF:RCo and VWF:CB were elevated in each patient. Five of six patients had ADAMTS13 levels below 45% (lower limit of normal) and three had levels of ~ 30%. Plasma levels of antibodies against ADAMTS13 were not detectable in any of the patients. Factor VIII and VWF levels were elevated at 279 (251–363) μ/dl and 350 (302–433) percent (median, IQR), respectively, and correlated with VTE in the study by Ladikou et al. [30].

Extracellular vesicles

Extracellular vesicles (EV) are lipid bilayer-encapsulated particles that are released by cells and transfer proteins, lipids, metabolites, nucleic acids, and organelles to other cells, often in tissues at distant sites of the body. *The release of VWF from platelets and ECs directly correlates with the release of EV from the same origins.* Various functions have been attributed to EV, including promotion of cell–cell signaling, elimination cellular waste or molecular recycling, and mediating host–pathogen interactions. EV are heterogenous in nature, ranging in size from 20 nm (nm) to 1 μm, and can be distinguished based on size with exosomes being on the low end and micro vesicles (previously referred to as microparticles) on the high end of the size range. EV are found in many biologic fluids such as plasma, urine, bile, saliva, bronchoalveolar lavage fluid, ascites, and lymph. In circulation, they can be released in response to pro-thrombotic conditions, inflammation, shear stress, and complement activation.

Platelets, megakaryocytes, leukocytes, endothelial cells and red blood cells all release EV, and may be influenced by EV-mediated effects. Platelet-derived EVs can originate from the plasma membrane of resting platelets or from alpha granules following platelet activation. Endothelial- and monocyte-derived EV express selectins, tissue factor, VWF, other coagulation factors, and negatively charged phosphatidyl serine that can promote thrombosis (reviewed in Fu) [45]. Circulating EV may also regulate inflammation and vascular permeability ([46]). Weibel-Palade derived small EV have also been found in circulation [47] and have a distinct molecular signature (CD36+/CD9−/CD81−) that distinguishes them from other small EV (CD36+/CD9+/CD81+).

Extracellular Vesicles and COVID-19

Severe acute respiratory syndrome, such as can occur with SARS-CoV-2 infection, has been associated with the development of autoantibodies [48] that may stimulate the classical pathway of complement activation to trigger the release of EV [49, 50]. In the context of COVID-19, it has been speculated that EV could regulate angiotensin converting enzyme 2, which is a key protein that is recognized by the SARS-CoV2 spike protein and mediates cell entry of the virus [51, 52]. By regulating proteins important for cellular entry of the SARS-CoV2 virus, EV might also regulate viral pathogenesis and disease severity (Fig. 1).

Infection with SARS-CoV-2 can lead to both endothelial dysfunction and dysregulated immune responses, conditions that are known to be associated with the generation of EV. In an analysis of plasma from 53 patients hospitalized with COVID-19 [53], Krishnamachary et al. characterized large (100–300 nm) and small (30–100 nm) EV, obtained by differential centrifugation of plasma at 20,000×*g* and 100,000×*g* respectively. The total number of small and large EV increased with disease severity, as did the mean particle size. Analysis of protein cargo in the large EV demonstrated distinct differences based on disease severity, with specific changes observed in the IL6 family and TNFα and TNF-receptor superfamily proteins. Prothrombotic factors within the large EV were also higher. In another study of 19 patients with COVID-19 pneumonia that were followed for 14 days for the development of severe respiratory failure [54], procoagulant phospholipid levels were higher in patients than healthy controls. No difference was observed in procoagulant phospholipid activity between patients with severe ($P0_2/Fi0_2 < 200$) and non-severe pneumonia, although

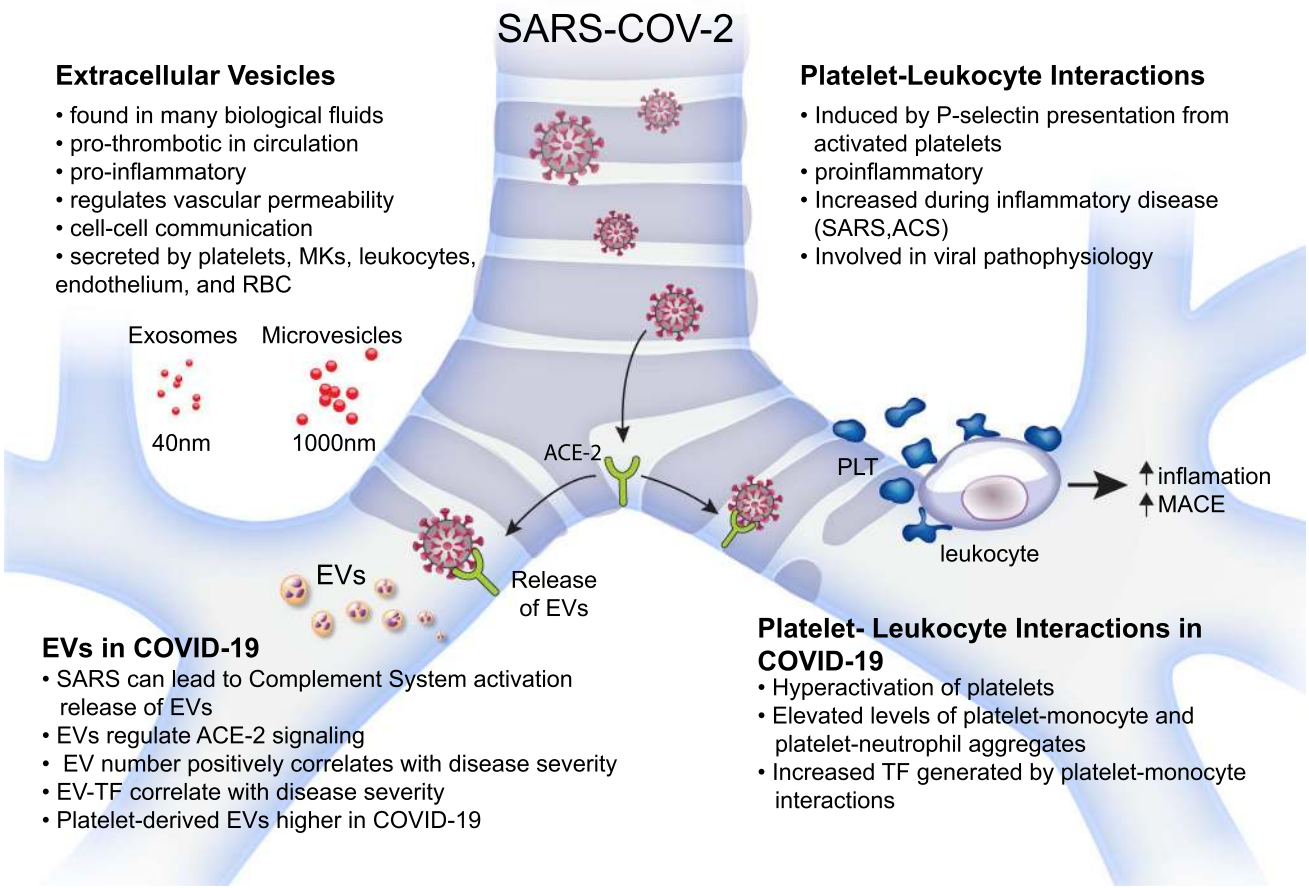

Fig. 1 The migration of inflammatory cells, specifically monocytes is responsible for a number of proinflammatory and prothrombotic steps, including tissue factor expression, cytokine production and along with neutrophils and their interaction with platelets, NETosis, protein expression, ROS production and recruitment of ULVWF to the endothelial cell surface. *NET* neutrophil extracellular traps, *VWF* von Willebrand Factor, *ROS* reactive oxygen species

higher procoagulant phospholipid levels on admission were associated with to severe respiratory failure, suggesting that they may be a marker for disease course.

In support of a role of EV in promoting thrombosis in COVID-19, Rosell et al. [55] demonstrated that circulating EV expressing tissue factor (TF) correlated with disease severity. They studied 100 patients with COVID-19 in comparison to 28 healthy controls. Levels of EV-associated TF activity were higher in COVID-19 patients than controls, higher in patients that required > 5L 0_2/min, and higher in non-survivors. Anticoagulation use did not affect levels of EV-associated TF activity nor did treatment with corticosteroids. EV-associated TF activity strongly correlated with D-dimer levels, a marker for thrombosis in this patient population. EV levels also correlated with prothrombin time, fibrinogen levels, plasmin-antiplasmin complexes, von Willebrand factor, and ADAMTS13. Guervilly et al. [56], also demonstrated that EV-associated TF activity increased with disease severity and correlated with leukocytes, D-dimer, and inflammatory parameters. This study further compared severe COVID patients to non-COVID septic shock patients and found that patients with COVID-19 had higher coagulopathy proles with significantly higher EV-TF activity.

Flow cytometry has been used to identify platelet-derived EV in plasma from patients with COVID-19 [57] using a cationic dye to bind the membrane, phalloidin to identify intact vesicles, and antibodies to platelet-specific CD41a (integrin αIIbβ3) or platelet and endothelial expressed CD31 (PECAM). Platelet-derived EV were higher in 2 cohorts of patients hospitalized with symptoms in April/May of 2020 (n = 23) or October/November of 2020 (n = 46) than in COVID-negative patients hospitalized during the same two time periods (n = 62) or healthy controls (n = 10). The results are in keeping with the findings of Taus et al. ([58]) who reported that platelet-derived vesicles, identified by expression of CD41 (integrin αIIb) and ranging in size from 100 to 1000 nm, were elevated in patients with patients with COVID-19 (n = 17) versus healthy controls (n = 22). Finally, Zaid et al. [25] noted increased numbers of platelet-derived

EV, identified by CD41, in both patients with severe (n = 44) and non-severe (n = 71) COVID-19 infection in comparison to healthy controls (n = 18). The total levels of platelet-derived EV were highest in the patient cohort with non-severe disease, and levels of phosphatidylserine-expressing platelet EV was only higher in patients with non-severe COVID-19.

Given the accumulated observed presence of EVs during COVID-19 and the long-known pro-inflammatory and pro-thrombotic nature of EVs, it is very likely that MVs play an important role in the progression and severity of the disease and therefore could be an important reporter or target for treatment in the future.

Platelet–leukocyte interactions

Under certain conditions, platelets are known to interact with a variety of cell types and tissues that they are exposed to circulating blood. Thus, the role that platelets play extends well beyond their well-known role in hemostasis. Among the cell types that platelets will interact with are leukocytes, primarily monocytes and neutrophils. These interactions are pro-inflammatory and platelet-leukocyte interactions have served as an indicator of in vivo platelet activation as well as a strong predictor of major adverse cardiovascular events (MACE). Circulating platelet-leukocyte aggregates and extracellular vesicles increase in animal models of lung injury [59]. Furthermore, platelet-leukocyte interactions are known to be increased during lung infections and antiplatelet therapy has been shown to drive down platelet-leukocyte interactions as well inflammatory biomarkers [60].

Platelet–leukocyte interactions in COVID-19

Platelet hyperactivation in patients with COVID-19 has been reported by several groups [24, 25, 58, 61]. Platelet–leukocyte interactions contribute to the pathophysiology of some viral infections and may play a similar role in SARS-CoV-2 infection. Expression of platelet P-selectin, an α-granule protein that undergoes translocation to the plasma membrane with platelet activation where is mediates platelet–leukocyte interactions, was higher patients with COVD-19. Platelet–monocyte and platelet–neutrophil aggregates as detected by flow cytometry were significantly higher in patients than healthy controls. In addition, an analysis of blood smears from COVID-19 patients revealed platelet engulfment by lymphocytes [58]. Leopold et al. [61] also found higher levels of platelet-neutrophil aggregates in patients hospitalized with COVID-19 (n = 35). In their study of COVID-19 patients within 72 h of ICU admission (n = 35), Hottz et al. [24] found higher levels of platelet-monocyte aggregates in ICU patients than asymptomatic/mildly symptomatic patients or healthy controls. In addition, TF expression by monocytes with attached platelets was significantly higher than in monocytes alone. Monocyte TF expression correlated with D-dimer levels, suggesting a potential role for platelet-stimulation of monocyte TF expression in thrombosis associated with COVID-19. Indeed, incubation of monocytes from healthy controls with platelets from patients with severe COVID-19 resulted in enhanced expression of monocyte TF in a manner that required platelet P-selectin and integrin αIIbβ3. In addition to promoting TF exposure and thrombin generation, platelet-leukocyte interactions can also contribute to neutrophil extracellular traps, cytokine release, generation of reactive oxygen species, and formation of novel transcellular metabolites of arachidonic acid [62]—each could promote inflammation and thrombosis in the setting of COVID-19.

Diagnostic assays

Peripheral blood

Peripheral blood diagnostic assays for VWF include initial screening, first level tests and second level tests. First level tests are widely available for the evaluation of patients with COVID-19. They include the measurement of plasma FVIII coagulant activity (FVIII:C), VWF antigen (VWF:Ag) and platelet-dependent VWF activity, the latter being commonly measured as ristocetin cofactor (VWF:RCo) that evaluates the ability of plasma VWF to agglutinate platelets in the presence of ristocetin. The ristocetin-triggered GPIbα binding (VWF:GPIbR) assay uses ristocetin and a GPIbα fragment captured by a monoclonal antibody coated onto an ELISA plate or latex or magnetic particles (for enhanced automated assays). The routine measurement of VWF antigen, activity or ADAMTS13 activity is not recommended at this time. The same is currently true for measuring EV and platelet- leukocyte aggregates.

Targeted treatment options

Scientific premise

ADAMTS13 cleaves VWF at its A2 domain. Accordingly, a therapy designed to inhibit the binding or catalytic activity of ADAMTS13 could prevent cleavage and the resulting down-stream effects. There are several challenges and considerations with such a strategy, including (1)VWF, under normal circumstances, is metabolized with a circulating half-life of 12–20 h, and (2) preventing either the binding or catalytic activity of ADAMTS13 could cause an excess of UL-VWF multimers and provoke the equivalent of thrombotic thrombocytopenic purpura (TTP)—a highly prothrombotic disorder [63]. Targeted therapies have not yet been tested in COVID-19.

VWF nanobody

Caplacizumab

Caplacizumab is a bivalent, humanized, single-variable domain immunoglobulin of nanobody that targets the A1 domain of VWF (reviewed in Knoebl) [64]. The drug inhibits the interaction between UL-VWF multimers and platelets. The drug is FDA-approved for the management of thrombotic thrombocytopenic purpura, including patients with refractory or recurrent disease [65].

Caplacizumab administration is subcutaneous after an initial intravenous loading dose. Cmax occurs within an hour [64] and binding to free VWF occurs rapidly and selectively. The caplacizumab-VWF complex circulates to the liver where it degrades within the reticuloendothelial system. Free (unbound) caplacizumab distributes to several organs where it is proteolytically degraded. The fraction of an administered dose recovered in the urine is small. The mean terminal half-life is 38.5 ± 22.2 h in healthy volunteers and is dose-proportional. A single 10 mg dose administered subcutaneously produced complete ristocetin-induced cofactor activity for 24 h [64]. It is currently approved for the treatment of thrombotic thrombocytopenic purpura.

Aptamers

Aptamers are single-stranded oligonucleotides that have theoretical advantages over other classes of therapeutic agents. They bind to their target with high affinity, in the low nanomolar to high picomolar range, like monoclonal antibodies.

BT200

BT200 is a pegylated RNA aptamer that inhibits binding of VWF to platelet GPIb [66]. Its target is the A1 domain and the circulating half-life exceeds 100 h following subcutaneous injection [67].

TAGX-0004

TAGX-0004 is a DNA aptamer developed to target the VWF A1 domain [68]. It contains DS—an artificial nucleic acid that has no complimenting base in nature, permitting unique 3D structures and enhancing its target binding affinity.

DTRI 031

DTRI-031 is a 36-nucleotide RNA aptamer that targets the A1 binding domain of VWF. It consists of a single stranded RNA. A matched reversal agent, DTRI-025, a sequence specific oligonucleotide that neutralizes the pharmacologic effect of DTRI-031 (< 5 min) is undergoing IND enabling studies. Preclinical testing with DTRI-031 has been undertaken [69, 70].

Cationic polymers

An ability to target thrombus-associated NETs bound to injured or disrupted ECs by VWF could represent a novel, yet viable option for the treatment of COVID-19-related thrombosis, particularly within the microvasculature. Preliminary evidence from Jain et al. [71] suggests that an approach to therapeutics based on binding of cationic polymers is feasible.

Recombinant ADAMTS13

The observed increase in VWF, coupled with decreased ADAMTS13 activity in COVID-19 lends itself to potential treatment options designed to restore VWF homeostasis. Turecek and colleagues incubated plasma samples obtained from 36 patients with severe COVID-19 with recombinant ADAMTS13 [72]. They observed a decrease in VWF activity and restoration of a normal pattern of multimer size.

Conclusions and future directions

The COVID-19 pandemic emphasizes the importance of understanding the pathobiology of disease, gauging the severity and natural history of disease with readily available and reproducible tools and making decisions about treatment and overall management according to the best available evidence. Circulating biomarkers of thrombosis, including VWF and extracellular vesicles have emerged and based on the information available may offer prognostic insights during the early phase of COVID-19. The question remaining is whether either represents a worthy target for therapy that will favorably influence clinical outcomes of patients with COVID-19.

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