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## **Communication from the Scientific Standardization Committees of the International Society on Thrombosis and Haemostasis on vascular endothelium-related biomarkers in disseminated intravascular coagulation**

Toshiaki Iba, Jerrold H. Levy, Jecko Thachil, Sophie Susen, Marcel Levi, Ecaterina Scarlatescu

<sup>1</sup>Department of Emergency and Disaster Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan <sup>2</sup>Department of Anesthesiology, Critical Care, and Surgery, Duke University School of Medicine, Durham, North Carolina, USA

<sup>3</sup>Department of Haematology, Manchester Royal Infirmary, Manchester, UK

<sup>4</sup>Department of Hematology and Transfusion, Lille University Hospital, Lille, France

<sup>5</sup>Department of Vascular Medicine, Amsterdam University Medical Center, the Netherlands and Department of Medicine, University College London Hospitals NHS Foundation Trust, and Cardio-metabolic Programme-NIHR UCLH/UCL BRC London, UK

<sup>6</sup>University of Medicine and Pharmacy “Carol Davila,” Bucharest and Department of Anaesthesia and Intensive Care, Fundeni Clinical Institute, Bucharest, Romania

### **Essentials**

In addition to the systemic activation in coagulation, endothelial dysfunction is an essential component of disseminated intravascular coagulation (DIC).

The significance of endothelial dysfunction is different depending on the underlying diseases, and it is most significant in sepsis-associated DIC.

The endothelium-related markers will make an earlier and a more accurate DIC diagnosis possible.

Further research is warranted to identify the ideal biomarker for endothelial dysfunction in DIC; however, antithrombin activity and von Willebrand factor are the possible candidates because those reflect severity and can be assessed in many clinical settings at the present time.

### **Abstract**

Disseminated intravascular coagulation (DIC) is not a disease criterion but a pathomechanistic process that accompanies various underlying diseases. According to the International Society on Thrombosis and Haemostasis definition, endothelial injury is an essential component in addition to systemic coagulation activation. Despite this definition, current diagnostic criteria for DIC do not include biomarkers for vascular endothelial injury. Endothelial cells are critical for hemostatic regulation because they produce various antithrombotic substances and express anticoagulant factors at the same time as facilitating coagulation, inflammatory reactions, platelet aggregation, and fibrinolysis with acute injury. Endothelial cells also exhibit various receptors, adhesion molecules, and the critical role of glycocalyx that regulates cellular interactions in thromboinflammation. For clinicians, biomarkers suitable for assessing endothelial injury are not readily available. Although we still do not have ideal biomarkers, antithrombin activity and von Willebrand factor can be candidates for the endothelium-related markers because those reflect the severity and are available in most clinical settings. Further, the dysfunction of endothelial cell in DIC arising from various underlying diseases is likely highly variable. For example, the

involvement of endothelial dysfunction is significant in sepsis-induced coagulopathy, while moderate in trauma-induced coagulopathy, and variable in hematologic malignancy-associated coagulopathy. Because of the complexity of disease status associated with DIC, further research searching clinically available endothelium-related biomarkers is expected to establish individualized diagnostic criteria and potential therapeutic approaches.

**KeyWords** adhesion molecules, antithrombin, disseminated intravascular coagulation, endothelial cell, glycocalyx

### **Introduction**

Disseminated intravascular coagulation (DIC) was first recognized as a mysterious complication of different underlying disease states, which result in thrombosis and/or bleeding [1]. The consensus definition of DIC was announced by the International Society on Thrombosis and Haemostasis (ISTH) in 2001 as “DIC is an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction [2].” At the same time, the ISTH released the “overt-DIC diagnostic criteria” for the definitive diagnosis, which identify the decompensated coagulation disorder by decreased platelet count, prolonged prothrombin time, increased fibrin-related marker, and decreased fibrinogen level [2] (Table). Although endothelial dysfunction is an essential condition for defining DIC, the overt-DIC diagnostic criteria include only the platelet count and coagulation tests and do not include endothelium-related markers despite the critical role of endothelial injury that can precede the coagulation disorder. The ability to monitor endotheliopathy would make early detection of DIC possible and facilitate potential therapeutic approaches [3], [4]

DIC, disseminated intravascular coagulation; ISTH, International Society on Thrombosis and Haemostasis; JSTH, Japanese Society on Thrombosis and Hemostasis; PF1+2, prothrombin fragment 1+2; SF, soluble fibrin; SIC, sepsis-induced coagulopathy; SOFA, sequential organ failure assessment; TAT, thrombin-antithrombin complex.

Total SOFA score is the sum of 4 items (respiratory SOFA, cardiovascular SOFA, hepatic SOFA, and renal SOFA).

DIC is still a refractory condition with high mortality. A recent nationwide study performed in Japan revealed that the mortality of septic patients with DIC was 24.8% [5]. Because DIC is not a disease category, but a mechanistic process that is involved in the disease's progress, detecting DIC in its earlier stage will be beneficial for predicting the outcomes and for the management of patients who are critically ill [6]. For that purpose, the ISTH recommends the screening of sepsis-induced coagulopathy (SIC), compensated phase of DIC, in patients with sepsis [7]. The diagnostic criteria for SIC consisted of platelet count, prothrombin time, and sequential organ failure assessment score [8], and again, the markers for endothelium-related markers are not included. The biomarkers reflecting endothelial injury are mostly used in research, and only seldom in clinical practice. In many clinical settings, even dealing with complex cases, there is still uncertainty about the possibility of assessing endothelial injury. However, some biomarkers reflecting functional changes of endothelium (endotheliopathy) are accessible for clinicians' use

and their measurement could improve the early detection and outcome prediction in DIC cases. Hereby the Scientific Standardization Committees (SSCs) on DIC and Perioperative and Critical Care thrombosis and hemostasis collaboratively evaluate the usefulness of markers for endotheliopathy in the management of DIC. The aim of this communication is to rethink the current coagulopathy-based DIC diagnosis and explore the possibility of improving it by including endothelium-related markers. For that purpose, we introduce the current knowledge on endothelium-related biomarkers. Previous research has revealed the critical roles of endotheliopathy in DIC pathogenesis, and assessment of endotheliopathy reportedly made early diagnosis and management possible. Although not ideal, some biomarkers are informative and accessible in various clinical settings. Another purpose is to facilitate research in this area to develop better endothelium-related biomarkers and more accessible techniques for their measurement.

### **Endothelial Cell-Related Biomarkers**

#### Adhesion molecules

Vascular endothelial (VE) cells interact with leukocytes and platelets and actively participate in thromboinflammation [9]. In this sequence, endothelial cells provide procoagulant, proadhesive, and proinflammatory conditions via expressing various adhesion molecules and facilitating microthrombus formation [10] (Figure). Leukocyte trafficking and platelet adhesion are predominantly observed in the postcapillary venules, where the blood flow is slow and sluggish. In the first step, selectin family adhesion molecules such as P- and E-selectin mediate tethering and rolling. In the next step, immunoglobulin superfamily adhesion molecules, such as intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1, execute the adhesion [11]. Regarding platelet adhesion, P-selectin-P-selectin glycoprotein ligand-1 linking is the first step for adhesion, followed by the integrin-platelet endothelial cell adhesion molecule link that contributes to scaffolding [12]. Other than those adhesion molecules that work for blood cell-endothelial interaction, endothelial cells express VE-cadherin. VE-cadherin is the transmembrane component of the endothelial adherens junction, which controls vascular permeability via regulating junctional strength [13]. The usefulness of measuring circulating levels of endothelial-derived adhesion molecules as biomarkers for endotheliopathy has been repeatedly reported [14, 15, 16]. Gando et al. [17] reported the levels of circulating adhesion molecules, including E-selectin, intercellular cell adhesion molecule-1, and vascular cell adhesion molecule-1, were significantly elevated in the patients with septic DIC. However, not many studies have examined the efficacy of measuring adhesion molecules in DIC.

#### Glycocalyx components

The surface of the vascular lumen is covered by the gel-like component, namely glycocalyx, that exhibits the antithrombotic, anti-inflammatory, and regulatory function of vascular permeability [10], [18]. The glycocalyx is quite vulnerable and is shed by various inflammatory mediators, such as heparanase, matrix metalloproteases, and reactive oxygen species [19]. Other than those, hyaluronidase, thrombin, elastase, plasminogen, and cathepsin B also degenerate the glycocalyx [20], and circulating glycocalyx components are considered as biomarkers for evaluating endothelial injury. The levels of glycocalyx components have been examined in various diseases, and their relations to endothelial injury were postulated [21], [22]. Syndecans are membrane-bound proteoglycans and are most

actively investigated. Nelson et al. [23] reported increased levels of syndecan-1 and syndecan-3 in critical conditions. The elevation of other fragments, such as hyaluronic acid and heparan sulfate, has also been examined, and their correlation to the severity of diseases is reported [24]; however, it is important to know syndecans are not specific markers for endothelium and they are expressed on the other cells, such as epithelial cells and plasmacytes [25], and further studies need to be done to determine their usefulness.

#### Extracellular vesicles

Extracellular vesicles are small particles enclosed by bilayer phospholipid membranes with a diameter of 0.03 to 5.00  $\mu\text{m}$  and act as mediators or messengers of mother cells [26]. Extracellular vesicles are released from almost all types of cells when they encounter various stimuli, and endothelial cell-derived extracellular vesicles are released under various vascular injuries [27]. In addition, other studies have reported the association between circulating levels of endothelial cell-derived extracellular vesicles and DIC [3],[28]. Activated endothelial cells propagate procoagulant activity by the enhanced release of procoagulant extracellular vesicles (mainly phosphatidyl serine-expressing vesicles) [29]. Furthermore, it was also reported that endothelial-derived extracellular vesicles activate the oxidative function of neutrophils and stimulate coagulation in sepsis [30]. In contrast, Lehner et al. [31] reported that endothelial-derived extracellular vesicles were unexpectedly low in patients with septic shock. These inconsistent results may owe to the performance of the flow cytometer. Simplicity and less cost are the essential conditions for the markers in diagnostic criteria. The measurement of extracellular vesicles is still costly and performed only in limited facilities, and improvements in these aspects are critical.

#### Plasminogen activator inhibitor 1

VE cells are involved not only in the regulation of coagulation but also in fibrinolysis by producing tissue-type plasminogen activator (tPA) and its inhibitor plasminogen activator inhibitor 1 (PAI-1). Numbers of experimental and clinical studies have reported that the plasma levels of PAI-1 but not tPA are associated with the severity and mortality of sepsis and DIC [32], [33]. It has been shown that the production of PAI-1 is affected by proinflammatory and anti-inflammatory cytokines, such as interleukin  $1\beta$ , interleukin 6, and transforming growth factor  $\beta$  and the elevated levels sustain longer [34]. Unbalanced fibrinolysis caused by excess production of PAI-1 is assumed to reflect the activation of endothelial cells and which is the typical feature of sepsis-associated DIC [35]. Since suppressed fibrinolysis finally leads to tissue malcirculation and organ dysfunction, PAI-1 is expected as a severity marker [36]. In healthy volunteers, endotoxin induces a rapid activation in the coagulation system and increased inflammatory cytokines within 120 minutes with a concurrent rise in tPA. This temporal activation in fibrinolysis is subsequently counteracted by a greater and sustained rise in PAI-1, leading to thrombogenesis [37]. Koyama et al. [38] measured various markers in sepsis and reported that thrombin-antithrombin complex (TAT), PAI-1, and protein C are useful for predicting DIC and poor outcomes. However, it should be kept in mind that PAI-1 is produced not only by endothelial cells but also produced by various cells, such as megakaryocytes and platelets [39]. Taken together, PAI-1 plays a major role in pathogenesis by suppressing fibrinolysis and can reflect the severity of DIC, but it is a nonspecific endothelium-related marker. By contrast, because tPA is exclusively produced by

endothelial cells, tPA/PAI-1 complex could be a better marker for endothelial activation [40].

### Antithrombin

Antithrombin, a serine protease inhibitor that inactivates factors VIIa, IXa, Xa, XIa, and IIa, is the most abundant and most important physiological anticoagulant [41]. The plasma levels of antithrombin activity can be measured by functional assays. Either antithrombin activity or anti-Xa activity is measured by the synthetic substrate method. The prognostic value of antithrombin activity for predicting morbidity and mortality has been repeatedly examined in sepsis-associated DIC, and superior performance than those of global coagulation tests was reported [42]. The supplementation of antithrombin for sepsis-associated DIC is recommended in the Japanese guidelines for sepsis management [43], and the measurement of the baseline antithrombin activity is commonly performed for selecting appropriate candidates as daily practice [42]. Similar to sepsis, low antithrombin levels are known to be associated with the risk of coagulopathy and thromboembolic complications in other diseases [44]. Although consumption by generated thrombin is suspected to be one of the mechanisms of decreased antithrombin activity, the level was irrelevant to TAT [45]. A much longer circulating half-life (a few days vs a few minutes) should be considered, but the normal antithrombin antigen level is 23.5 to 33.5 mg/dL and TAT level in DIC is reportedly 20 to 50 ng/mL, which means only a very small portion of antithrombin is consumed for neutralizing thrombin. After all, although the lower plasma concentration of TAT than antithrombin should be taken into account for the quick metabolism of TAT from circulation, the TAT level is still lower than the antithrombin level. However, it should also be considered that antithrombin makes complexes not only with thrombin but also with many other coagulation factors that are not routinely measured. Whereas, because plasma antithrombin levels before and after antithrombin administration are associated with serum albumin levels, antithrombin activity is considered to be determined by vascular permeability [46]. Matsumoto et al. [47] demonstrated that decreased antithrombin activity was associated with circulating thrombomodulin and serum albumin levels but not with TAT also in patients with trauma. In addition, Bernet-Buettiker et al. [48] reported increased antithrombin activity in chylothorax in patients with chylothorax and suggested the levels of antithrombin are regulated by capillary leakage. It is also known that the antithrombin levels decrease most significantly in patients with septic DIC, where endothelial dysfunction is most significant. Meanwhile, the levels are maintained best in patients with DIC with hematologic malignancy where the endothelial dysfunction is least significant, and the levels are in between in trauma-induced patients with DIC.

It also has to be kept in mind that a large part of antithrombin is known to bind to heparan sulfate of the glycocalyx at the endothelial surface, and when the endothelium is injured, the glycocalyx will be disrupted, and this may affect plasma levels of antithrombin. It was reported that the antithrombin activity was maintained at around 80% in septic patients without organ dysfunction, but the activity decreased to approximately 60% in patients with organ dysfunction, and the level was lower, and 40% in patients with DIC, and the decrease in activity preceded the presence of DIC [49]. Others have reported that the diagnostic value of an area under the receiver operating characteristic curve of the antithrombin activity exceeded 0.8 for the prediction of patient outcome [50], [51]. In addition, Choi et al. [52] reported a significant correlation between antithrombin level and DIC score in patients with

sepsis. These results indicate that antithrombin activity is a good biomarker of endothelial dysfunction and is useful for predicting the morbidity and mortality of sepsis with DIC. Although most of the studies were performed in septic DIC, antithrombin activity is included in the diagnostic criteria for DIC newly proposed by the Japanese Society on Thrombosis and Hemostasis [53]. Nevertheless, it should be cautioned that antithrombin is not a specific marker of endothelial dysfunction, and the level is also affected by hepatic function.

#### Thrombomodulin

Thrombomodulin is a cofactor for thrombin-catalyzed activation of protein C [54]. It is expressed on the endothelial surface and plays important roles in both regulating thrombosis and inflammation. This molecule switches the thrombotic activity of thrombin to antithrombotic through activating protein C [55]. In addition, its unique structure lectin-like domain expresses anti-inflammatory and cytoprotective properties [56], [57]. It is known that soluble thrombomodulin, fragments of the extracellular region of membrane-bound thrombomodulin cleaved by leukocyte-derived proteases or metalloproteases, are released into the circulation in sepsis and other inflammatory diseases [55]. The shed extracellular domain of thrombomodulin can be detected in plasma and urine, and their levels are known to increase several-fold higher in various inflammatory and thrombotic conditions, such as sepsis, trauma, acute respiratory distress syndrome, pulmonary embolism, and DIC [58], [59]. Multiple studies have reported the usefulness of measuring soluble thrombomodulin to evaluate the severity of DIC [60], [61]. However, the increase in soluble thrombomodulin does not simply reflect endotheliopathy. Because the synthesis of thrombomodulin is strongly downregulated at the messenger RNA level during sepsis, the decrease in thrombomodulin on the cell surface is not only because of protease-mediated shedding but also because of decreased expression. Unfortunately, the measurement is not commonly performed in practice, and widespread of testing is expected.

#### von Willebrand factor

von Willebrand factor (VWF) is an essential hemostatic protein that is synthesized in endothelial cells and stored in Weibel–Palade bodies and released into circulation under various stimuli. Nevertheless, VWF is not an endothelium-specific marker, it is also stored in alpha granules of platelets, and platelet-derived VWF is known to play a major role in the formation of platelet-rich thrombi. Circulating VWF contributes to has a major role in hemostasis as well as in the pathogenesis of DIC. The primary function of VWF is the promotion of platelet adhesion by anchoring them to the subendothelial matrix [62]. With respect to the utility as biomarkers, Jang et al. [63] reported that platelet count/VWF antigen ratio was a strong prognostic marker in DIC. In contrast, Walborn et al. [64] compared the performance of markers of endothelial activation (such as endocan, angiopoietin-2 [Ang-2], and VWF), natural anticoagulants (tissue factor pathway inhibitor and protein C), and damage-associated factors (high mobility group box 1) for predicting the severity and outcome of sepsis-associated DIC. As a result, VWF did not demonstrate a better performance compared with the other biomarkers. However, Chang [65] ] reported the role of the microthrombus composed of platelets and unusually large VWF multimers (UL-VWF) in the pathogenesis of DIC. The critical role of UL-VWF is also emphasized in the development of microthrombosis in COVID-19–associated coagulopathy [66]. As a reminder, UL-VWF newly presented on the endothelial

surface by exocytosis is strongly bioactive; however, the assay for VWF activity and VWF antigen may not correlate to the bioactivity of UL-VWF in the vessels. Also, UL-VWF is proteolytically cleaved on the endothelial cells by ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13), but the activity of ADAMTS13 decreased significantly in DIC [67]. Therefore, the significance of VWF should always be discussed with the presence of UL-VWF and ADAMTS13 activity. Although not much data has been accumulated, because the measurements of VWF and ADAMTS-13 are also important to differentiate thrombotic thrombocytopenic purpura, the accessibility to those tests will increase. It is also important to know that there is a link between activated coagulation and VWF levels since the release of VWF is stimulated by thrombin. After all, although VWF cannot be determined as endothelium-specific biomarker, because its accessibility is relatively good, it can potentially be valuable to measure as endothelium and thrombin-related marker in DIC.

### Angiopoietin 2

Similar to VWF, angiopoietin 2 is stored in the Weibel–Palade bodies of the endothelial cells and released into circulation. Thus, the plasma levels of angiopoietin are expected as a biomarker for endothelial activation. Angiopoietin 2 competitively binds to the common receptor as angiopoietin 1 and serves as an antagonist of angiopoietin 1 [68]. Opposite to the anti-inflammatory, anticoagulatory, and antiapoptotic actions induced by angiopoietin 1, angiopoietin 2 induces prothrombotic responses by expressing endothelial tissue factor, and phosphatidylserine exposure in sepsis that leads to DIC [69]. Angiotensin 2 is also known to increase endothelial permeability, and therefore, it is considered a critical factor in multiple organ dysfunction [70]. A clinical study demonstrated that higher levels of serum angiopoietin 2 were associated with decreased survival in sepsis [71], and another study has shown the association with a higher likelihood of DIC [72]. Concerning the clinical usefulness, not many studies have been done, and further study is warranted.

### Endothelial dysfunction in various types of DIC

Although endotheliopathy is recognized in every type of DIC, the impact differs among the underlying diseases. The impact is most significant in sepsis-associated DIC, and all the aforementioned mechanisms are relevant to the pathogenesis of sepsis-associated DIC. Inflammation is a fundamental host defense against infection, and proinflammatory cytokines, immune cells, and activated coagulation stimulate endothelial cells and convert their antithrombotic property to thrombotic and hemostatic [2], [5], [65].

This type of DIC is called “thrombotic-type” or “suppressed fibrinolysis type” DIC, and endotheliopathy takes the lead in the pathogenesis of DIC. Antithrombin levels decreased considerably even before the onset of DIC, and the decreased levels are correlated to morbidity and mortality [43, 44, 45, 46, 47, 48, 49]. In contrast, the role of endothelial dysfunction is less significant in “bleeding type” or “enhanced fibrinolysis type” DIC. For example, acute promyelocytic leukemia is frequently complicated by DIC, and high levels of annexin II on leukemic cells are responsible for the upregulated fibrinolytic system [73]. Because leukemic cells express tissue factor concurrently, they activate coagulation that causes DIC. The relevance of endothelial dysfunction in acute promyelocytic leukemia is considered to be less significant but generated thrombin affects the endothelial function during DIC.



Because the endotheliopathy seen in this type of DIC is thrombin mediated, it is recognized as passive injury. Finally, endothelial cell dysfunction in trauma-induced coagulopathy is the type in between. The typical feature of trauma-induced coagulopathy is impaired hemostasis, and massive transfusion is often demanded. The coagulopathy is triggered by thrombin generation because of tissue injury, which also damages endothelium and leads to its unique hyperfibrinolytic condition [74]. In the latter types of DIC/coagulopathy, the merit of measuring endotheliopathy markers will be less significant.

### **Summary**

Because endothelial dysfunction is an essential part of DIC pathophysiology, it is not surprising that endothelium-related markers correlate with the development or severity of DIC. Various underlying diseases are known to develop DIC, and notably in some diseases, such as sepsis and COVID-19, endothelial dysfunction accounts for the causal factors; in contrast, endothelial dysfunction can occur as a result of coagulation activation in other diseases, such as trauma and hematologic malignancy. In such situations, their value may be less significant. The inconsistent value is explained by the different pathogeneses of DIC subtypes. It is generally accepted that the involvement of endotheliopathy is significant in SIC, and it is moderate in trauma-induced coagulopathy and mild in hematologic malignancy-associated coagulopathy. In diseases in which endothelial dysfunction precedes coagulation disorder, monitoring endothelial dysfunction may be useful.

As for the addition of new biomarkers to the diagnostic criteria of DIC, because DIC is a common complication, the diagnosis must be made using readily available laboratory tests. However, most of the tests introduced in this communication are still performed for research purposes at present. From this viewpoint, we need to continue searching for the ideal endothelium-related biomarkers. In summary, although members of DIC and Perioperative and Critical Care SSCs recognize that there is no endothelium-specific marker, we assume antithrombin activity and VWF for the candidates of endothelium-related markers in DIC because these biomarkers showed better performance in terms of severity and outcome prediction. In addition, they are widely and practically used, at least in certain countries. Finally, both SSCs recommend activating the research on endothelium-related markers.

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This issue is discussed in the joint session of the Scientific Standardization Committees (SSCs) on DIC and perioperative and critical care thrombosis and hemostasis in the ISTH annual meeting in 2022.

### **Declaration of competing interest statement**

T.I. participated on advisory boards of Japan Blood Products Organization, Asahi Kasei Pharmaceuticals, and Toray Medical. J.H.L. serves on the Steering or Advisory Committees for Instrumentation Laboratories, Merck, Octapharma. S.S. serves on the Steering or Advisory Committees for Sobi, Roche, CSL Behring, NovoNordisk, Takeda, LFB, and BioMarin. M.L. has received grants and has participated in advisory boards of NovoNordisk, Eli Lilly, Asahi Kasei Pharmaceuticals America, and Johnson & Johnson. The other author has no conflict of interest.

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