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Review

Microparticles: new light shed on the understanding of venous thromboembolism

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Microparticles are small membrane fragments shed primarily from blood and endothelial cells during either activation or apoptosis. There is mounting evidence suggesting that microparticles perform a large array of biological functions and contribute to various diseases. Of these disease processes, a significant link has been established between microparticles and venous thromboembolism. Advances in research on the role of microparticles in thrombosis have yielded crucial insights into possible mechanisms, diagnoses and therapeutic targets of venous thromboembolism. In this review, we discuss the definition and properties of microparticles and venous thromboembolism, provide a synopsis of the evidence detailing the contributions of microparticles to venous thromboembolism, and propose potential mechanisms, by which venous thromboembolism occurs. Moreover, we illustrate a possible role of microparticles in cancer-related venous thromboembolism.

Keywords: microparticle; blood cell; endothelial cell; venous thromboembolism; intercellular communication; inflammation; cancer

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Introduction

Significant progress has been made recently in our understanding of microparticles (MPs). MPs (also called microvesicles) are small (0.1–1 μm) membrane fragments shed primarily from blood and endothelial cells (ECs) during either activation or apoptosis. They are detected and characterized on the basis of their heterogeneous compositions, which reflect their cellular origins[1].

Despite circulating in both blood and biological fluids in healthy individuals, MPs have been implicated in a wide spectrum of diseases, including cardiovascular disease[2–4], diabetes[5], inflammatory states[6, 7], sepsis[8], antiphospholipid antibody syndrome[9, 10], thrombotic thrombocytopenic purpura[11] and cancer[12]. Many of these conditions are characterized by a hypercoagulable state, which may lead to thrombosis. Additionally, an important link between MPs and venous thromboembolism (VTE) has been established via the collection of large amounts of new evidence, which may shed light on novel interpretations of how VTE occurs and represent possible therapeutic approaches for treating the phenomenon. This review discusses relevant studies and highlights the contributions of MPs to VTE, as well as the potential role played by MPs in cancer.

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Definition and properties

MP formation, composition, and function

A wide variety of normal cells (blood cells and ECs) generate MPs, as do malignant cells. Both chemical (*eg*, cytokines, thrombin, cytotoxic chemotherapy[13], cholesterol enrichment[14] and tobacco smoke exposure[15]) and physical stimulation (*eg*, shear stress, hypoxia and stimuli[16–18]) trigger MP release. Additionally, MPs participate in cell apoptosis.

Cell activation leads to elevated levels of intracellular Ca^{2+}, resulting in the disruption of membrane asymmetry and in increased exposure of phosphatidylserine (PS), which resides in the inner membrane, to the cell surface, as well as membrane blebbing and subsequent MP shedding[19]. Although calcium entry and the following activities play a putative role in MP formation, the exact mechanisms that govern MP formation remain unclear. Previous studies have shown that mitogen-activated protein kinases (MAPKs) are involved in the process of MP formation through the investigation of MPs produced by human macrophages when exposed to tobacco smoke[20]. Additional studies are needed to better outline mechanisms of MP formation and to investigate whether there

are differences in the mechanisms underlying MP formation, depending on whether this process is associated with activation or apoptosis.

As sealed membrane vesicles, MPs not only bear antigens indicative of their cellular origin but also retain biological molecules. Considered to be key features of a ubiquitous and highly organized system of cell communication and intercellular material transport[21–24], MPs may induce cell signaling[25] and regulate many pathophysiological processes[26] by harboring inflammatory components (LpA and cytokines), growth factors (TGF beta and VEGF) and proteases (uPA and MMP)[25, 27], in addition to altering the activities of tissue factor (TF)[28] and PS.

MP detection and measurement

Currently, flow cytometry is the most common method of quantifying MPs. MPs may also be detected using antibody capture assays, such as enzyme-linked immunosorbent assay[29], or modified capture protocols that detect functional properties of MPs. Results of MP functional assays and the number of MPs identified by flow cytometry from the same sample are often uncorrelated. A pitfall of flow cytometry is that it has limited forward scatter sensitivity; therefore, standard flow cytometers measure only a small portion of the MP population. Fortunately, recent technological improvements resulting in the advent of high sensitivity flow cytometry have made it possible to measure small-size MPs, which represents a new opportunity to utilize flow cytometry for MP measurement[30]. We believe that flow cytometry will remain a mainstream approach for testing MP levels in the near future; however, a standardized testing protocol must be established. To accomplish this, research regarding sources of variability within the assay is necessary. Additionally, new techniques, such as laser-induced nanotracking[31], atomic force microscopy[32], and dynamic light scattering, have been developed. These advances represent exciting possibilities for MP measurement.

Information about a series of recent studies[33–38] investigating MPs in patients with VTE is included in this article (Table 1 and 2). Most of the studies demonstrated an increase in different types of MPs, but some did not, resulting in an inconsistency among the results of these investigations. There may be several reasons for these discrepancies. One reason may be different times of blood sampling. According to Table 1 and 2, blood samples were collected in the setting of acute events in the studies by Chirinos *et al*, Ye *et al* and Bai *et al*[34, 36–38]. However, Bucciarelli *et al*[35] collected blood samples after the first episode of VTE (<5 years) and long (>3 months) after any possible trigger events, such as surgery or pregnancy, which may affect MP levels. Ay *et al*[33] studied patients suffering from recurrent VTE and collected their blood at least 3 months after each patient's most recent VTE event. Studies that collected blood samples in the setting of an acute event demonstrated elevated MP levels, which are associated with VTE, but the study by Ay *et al*[33], which collected blood long after each acute episode, failed to demonstrate these findings. However, the study by Bucciarelli *et al*[35] revealed that an increased relative risk of VTE was still observed in association with high MP levels when patients were tested soon after their VTE, although this information was excluded from their analysis of VTE risk. Therefore, the effects of acute disease processes on MP formation remain unclear. Another reason for the inconsistencies may be the different methods utilized in each study. As previously mentioned, the studies by Bucciarelli *et al*[35] and Ay *et al*[33] presented different outcomes following the investigation of blood samples collected at times long after an acute event. Flow cytometry was used to evaluate MP levels in the former[35], whereas functional assays were utilized for the latter project[33]. Unlike flow cytometry, which provides details regarding size, number and origin of MPs, functional assays provide information on MP activity. Therefore, we speculated that the utilization of different analysis methods may have played a critical role in the final results of each study. A third reason for the discrepant results may be differences in the markers used to identify MPs because several types were used to identify MPs. In the three studies in question[34, 37, 38], there were three types of markers used to assess the MPs (CD31+, CD42b+, CD41a+, and glycoprotein Ib+), which may explain the conflicting results of these studies. The discordant findings caused by the utilization of different techniques

Table 1. Studies measuring microparticles in non-acute conditions in patients with venous thromboembolism.

Study	Subjects	Study/control Number	Time of blood sampling	Method	MPs detected	Specific marker	Results
Ay *et al*[33]	Patients with recurrent VTE	116/129	>3 months after the most recent event of VTE	Functional assay	CPMPs	Annexin V+	↑
Bucciarelli *et al*[35]	Patients after a first episode of VTE	186/418	After the first episode of VTE(<5 years) and far (>3 months) from triggering events	Flow cytometry	Total MPs	Annexin V+	↑
					PMP	Annexin V+, CD41+	↑
					MPTF	Annexin V+, CD142+	↑

↑ Means a significant increase of MPs in patients with VTE compared with healthy controls; – means no significant difference found between patients with VTE and healthy controls.

CPMPs, circulating procoagulant microparticles; MP, microparticle; MPTF, tissue factor-bearing microparticle; PMP, platelet microparticle; VTE, venous thromboembolism.

Table 2. Studies measuring microparticles in acute conditions in patients with venous thromboembolism.

Study	Subjects	Study/ control Number	Time of blood sampling	Method	MPs detected	Specific marker	Results
Bai *et al*[34]	Patients with acute PE	45/45	In acute condition of VTE	Functional assay	CPMP	Annexin V+	↑
					PMP	Glycoprotein Ib+	↑
					EMP	CD31+	–
Campello *et al*[36]	Patients with a first episode of unproked VTE	30/90	In acute condition of VTE	Flow cytometry	EMP	Annexin V+, CD146	↑
					PMP	Annexin V+, CD61	↑
					MPTF	Annexin V+, mAb anti-TF	↑
Chirinos *et al*[37]	Patients with acute VTE	25/25	In acute condition of VTE	Flow cytometry	EMP	CD31+, CD42b-; CD62E	↑
					PMP	CD31+, CD42b+	–
					Platelet activation	CD62P	↑
					Leukocyte activation	CD11b	↑
					EMP-monocyte conjugates	CD45+, CD62E	↑
					Platelet-leukocyte conjugates	CD45+, CD41+	↑
Ye *et al*[$] [38]	Patients with acute recurrent VTE	25/25	In acute condition of VTE	Flow cytometry	Total MP/MPTF	Annexin V+/Annexin V+, 4507CJ+	↑/↑
					Monocyte-derived MP/MPTF	CD14+/CD14+, 4507CJ+	↑/↑
					PMP/MPTF from platelets	CD41a+/CD41a+, 4507CJ+	–/↑
					EMP/MPTF from endothelial cells	CD144+/CD144+, 4507CJ+	↑/↑
					Erythrocyte-derived MP/MPTF	CD235a+/CD235a+, 4507CJ+	–/–

↑ means a significant increase of MPs in patients with VTE compared with healthy controls; – means no significant difference found between patients with VTE and healthy controls.

[$] Annexin V+/Annexin V+, 4507CJ+ means Annexin V+ for total MP, Annexin V+, 4507CJ+ for total MPTF, respectively. So do the rest expression in the study of Ye *et al*.

CPMP, circulating procoagulant microparticle; EMP, endothelial microparticle; MP, microparticle; MPTF, tissue factor-bearing microparticle; PE, pulmonary embolism; PMP, platelet microparticle; VTE, venous thromboembolism.

and different methodological approaches have blurred our understanding of MP activities and increased our craving for standardized assays capable of generating results with a high degree of reproducibility.

Contribution of MPs to VTE

VTE is a multifactorial disease that includes deep venous thrombosis (DVT) and pulmonary embolism (PE). Increasing evidence points to elevated levels of different phenotypes of MPs in patients with VTE, suggesting that MPs play an important role in the pathophysiology of VTE[34–38]. It is noteworthy that inflammation and thrombosis are coupled via common activation pathways and feedback regulation systems because this establishes a relationship between VTE and inflammation. The functional interplay among ECs, platelets, inflammatory cells and MPs deduced by studying those cells may play an indispensable role in VTE. We have elucidated the effects of MPs on thrombogenesis in three ways based on previous studies: the expression of TF and exposure of PS, MP-induced intercellular communication, and MP involvement in crosstalk between inflammation and VTE. Further research is required, however, to determine whether circulating concentrations of MPs are sufficient to facilitate thrombus formation and mediate inflammation.

Procoagulant properties of MP: the expression of TF and the exposure of PS

Circulating monocytes, MPs, and activated endothelium produce a procoagulant protein, TF, under pathological conditions[39, 40]. These intravascular sources of TF may trigger the formation of venous clots via the extrinsic pathway of the blood coagulation cascade. Although activated monocytes and tumor cells are the primary sources of TF-positive MPs in the circulation[41], TF has been identified on leukocyte MPs, endothelial microparticles (EMPs) and platelet microparticles (PMPs)[9, 11, 42–46]. Additionally, it is interesting to note that the density of active TF on microvesicles (MVs) was higher than the protein's density on their parental cells[47], which supports the hypothesis that MP formation is not entirely a random process and that MPs are effective products made in response to a changing environment.

The idea that TF is a potent trigger of VTE is promising, although accurate mechanisms detailing its involvement in the coagulation system have not been clearly elucidated. This important role has been consigned to TF not only as a result of its over-expression in the setting of thrombotic processes but also because a genetic deficiency in TF in hematopoietic cells and myeloid cells dramatically reduces the incidence of venous thrombosis, which indicates that TF expression by leu-

kocytes and leukocyte derived MVs initiate thrombosis; this has been suggested by recent studies using a mouse inferior vena cava stenosis model[48]. TF-positive MPs may therefore serve as a critical source of TF and play an active role in the initiation of VTE.

The exposure of PS is another trait of MP formation. The anionic PS serves as a catalytic surface for the assembly of the prothrombinase complex[49]. Moreover, phospholipids on the surface of MPs derived from platelets and ECs provide a number of binding sites for factors Va, VIII, IXa, and IIa[49–52], allowing for concentrations necessary to achieve optimal thrombin generation and efficient hemostasis[53]. Therefore, PS amplifies the procoagulant activity of TF and contributes to the propagation of the coagulation cascade.

MP-induced intercellular communication

Studies in several settings[14, 15, 45] have demonstrated that only a minority of microvesicles are TF-positive. Recent studies suggest that microvesicles are significant mediators of intercellular communication under physiologic and pathologic conditions[54–56]. Our previous project indicated that MPs from cholesterol-loaded human cells function as novel carriers of damage-associated molecular patterns[57]. Therefore, microvesicles may arise from and mediate novel physiologic and pathologic effects independent of coagulation. We propose that MPs may play this role by acting as mediators of endothelial dysfunction and serving as shuttles promoting cellular cross-talk[58]. In this study, we focused on MP-induced intercellular communication and the possible role of this mediation in the underlying processes of VTE formation.

EMP-induced intercellular communication

The most common site of thrombus formation in humans is the valve pocket sinus, which results from turbulent flow and hypoxia[59]. The endothelium at this location may be activated by either hypoxia or inflammatory mediators and subsequently expresses the adhesion proteins P-selectin, E-selectin, and von Willebrand factor (vWF), which capture leukocytes, platelets, and MPs[60, 61]. Additionally, activated ECs release EMPs. In addition to the aforementioned procoagulant properties of MPs, which are related to TF expression and PS exposure, EMPs may lead directly to the development of endothelial dysfunction. It has been demonstrated that patients with VTE show marked elevations in EMP identified by CD31+/CD42b-(EMP_{31}) (2193 *vs* 383 counts/μL; P=0.003), E-selectin (EMP_{62E}) (368 *vs* 223 counts/μL; P=0.001), and EMP-monocyte conjugates (3.3% *vs* 2.5%; P=0.002)[37]. These findings support those of prior studies suggesting that the release of EMP and its subsequent binding to monocytes are key events in thrombogenesis[62, 63]. Additionally, *in vitro* experiments have demonstrated that EMPs promote and stabilize platelet aggregates by expressing ultralarge vWF[64] and that oxidized phospholipids in EMPs exposed to oxidative stress may be particularly active in mediating both monocyte adherence to ECs and the activation of neutrophils[65, 66].

Intercellular communication associated with MPs derived from leukocytes

Marked leukocyte activation (13.9 *vs* 7.7 U for CD11b; P=0.004) in the setting of VTE was observed in a recent study[37]. MPs derived from leukocytes express P-selectin glycoprotein ligand-1 (PSGL-1), which is inherited from their parental cells and interacts with a key endothelial cell receptor, P-selectin. Reduced thrombosis was demonstrated via the inhibition and deficiency of P-selectin[67–69], which indicate that the interaction between PSGL-1 and P-selectin, specifically the binding of leukocyte-derived MPs to the activated endothelium, is involved in thrombogenesis. These studies also suggest that blocking the binding of leukocytes and MPs to activated EC may provide a novel strategy for preventing VTE. Moreover, it has been suggested that MPs shed by leukocytes stimulate cytokine release and the induction of TFs in ECs by activating a signaling pathway involving the tyrosine phosphorylation of c-Jun NH_2-terminal kinase-1, which may lead to increased proinflammatory and procoagulant activity in ECs[70].

Leukocyte-derived MPs also contribute to the development of thrombi through the recruitment of platelets and the accumulation of TF. ECs and platelets express P-selectin. As demonstrated previously, monocytes and macrophages are primary sources of circulating TF via the shedding of TF-bearing MPs[41]. These MPs, which have PSGP-1 on their surface, participate in platelet thrombus formation not merely by binding to P-selectin on activated platelets[67] but by fusing with these platelets via PSGL-1, transferring lipids and proteins, including TF, into their plasma membranes[71].

PMP-induced intercellular communication

There was a dramatic increase in platelet activation (35.2 *vs* 5.0 fluorescence intensity units for P-selectin; P<0.0001) in the setting of VTE, which was found in the recent study by Chirinos *et al*[37]. Likewise, elevated PMPs were detected in patients with VTE in two other studies[34, 35], and a strong correlation was found between total MPs and PMPs (ρ=0.99, P<0.0001) and between PMPs and TF-bearing MPs (ρ=0.94, P<0.0001) in the study by Bucciarelli *et al*[35]. An increased amount of TF-bearing MPs of platelet origin (P=0.004) was found in patients with acute recurrent VTE, whereas no significant differences in total PMPs (P=0.062) were found when these results were compared with similar studies in healthy subjects[38]. These findings indicate an association among PMPs and TF-bearing MPs of platelet origin and VTE. However, there is unfortunately no direct evidence corroborating the existence of PMP-induced intercellular communication *in vivo*. However, a series of *in vitro* studies have suggested these putative effects of PMP based on interactions with other cells. These studies demonstrated that MPs released by aggregating platelets may facilitate platelet activation and EC activation via the transcellular delivery of arachidonic acid or other mediators[72, 73]. Moreover, PMP binds to and activates neutrophils *in vitro*[74]. Further *in vivo* studies are necessary to address the role of PMP in this process.

MP involvement in crosstalk between inflammation and VTE

Inflammation and hemostasis share an interactive relationship because they are linked via common activation pathways and feedback regulation systems. Emerging evidence supports the idea that MPs may play a role in crosstalk between inflammation and thrombosis. As suggested in the recent study by Chirinos *et al*[37], patients with VTE demonstrated dramatic elevations in their levels of MPs and conjugates between platelets and leukocytes (PLC) (61.7% *vs* 39.6%; P=0.01). They also found that there was a strong correlation between PLC and the degree of leukocyte activation (r=0.74; P<0.0001). Their results support the idea that the formation of PLC regulates leukocyte activation and participates in linking thrombosis to inflammation. This link may be related to the increased levels of MPs because EMPs, like PMPs, have been shown to function as vectors for many inflammatory mediators[73]. Additionally, leukocyte-derived MPs may precipitate increased proinflammatory and procoagulant activity by interacting with other cells by aforementioned means.

In summarizing the functions of MPs, we propose a mechanism for VTE according to the above points (Figure 1). ECs located at the valve pocket sinus may be activated by either hypoxia or inflammatory mediators. Activated ECs express the adhesion proteins, P-selectin, E-selectin, and vWF, which capture leukocytes, platelets and MPs, and release EMPs. Circulating leukocytes bind to ECs and EMPs via P-selectin and E-selectin, and platelets bind to ECs via vWF. These bindings activate leukocytes, inducing TF expression on leukocytes and triggering the shedding of TF-bearing MPs from leukocytes, which may interact with ECs and activated platelets. The high concentration of TF and the wide catalytic surface for the assembly of the prothrombinase enzyme complex trigger thrombus formation. Additionally, EMPs may express vWF to stabilize platelet aggregation (Figure 1).

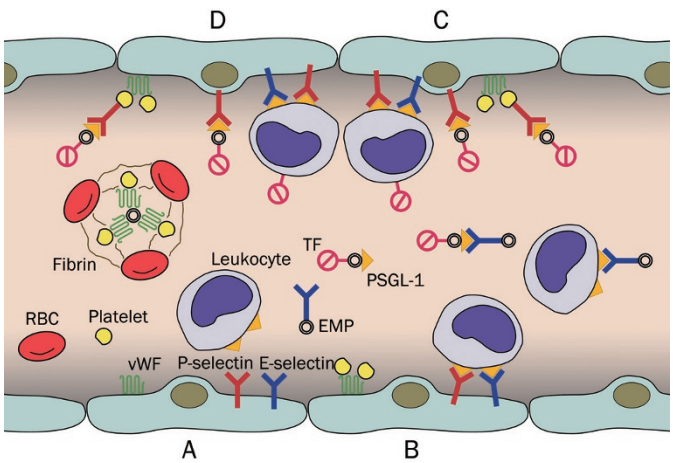

Figure 1. The role of microparticles in the mechanism of venous thromboembolism. (A) Activated endothelial cells express P-selectin, E-selectin, and vWF and release EMPs; (B) Leukocytes bind to endothelial cells and EMPs via P-selectin and E-selectin; platelets bind to endothelial cells via vWF; (C) These bindings activate leukocytes that induce TF expression and shedding of TF-bearing EMPs, which interact with endothelial cells and activated platelets; (D) The high concentration of TF and wide catalytic surface contribute to thrombosis; EMPs express vWF to stabilize platelet aggregation. EMPs, endothelial microparticles; PSGL-1, P-selectin glycoprotein ligand-1; TF, tissue factor; vWF, von Willebrand factor.

Contribution of MPs to cancer-related VTE

There is a strong correlation between VTE and cancer regardless of cancer types and stages. Given the crucial role that MPs play in the process of VTE, the relationship between MPs and cancer has been the subject of recent studies and review articles, which have offered novel interpretations of the interaction among MPs, thrombosis and cancer[36, 75–79].

A study investigating endothelial cells, platelets, and TF-positive MPs in cancer patients with and without VTE demonstrated statistically significant elevations in TF-positive MP plasma levels (1019±656 MPs/μL) in cancer patients with VTE compared to cancer patients without VTE (755±391 MPs/μL, P=0.002)[36]. Nevertheless, multivariate analysis failed to show a significant association between elevated TF-positive MPs and VTE in cancer patients[36]. Another study demonstrated that patients with different types of cancer who also developed VTE demonstrated higher numbers of circulating MPs and particularly higher activity levels of TF-bearing MPs than cancer patients without VTE[80], a finding confirmed by three other studies that used flow cytometry, chromogenic assays, and prothrombinase assays[77–79]. Collectively, these studies have corroborated the existence of an association between thrombosis and TF-bearing MP expression in cancer. However, this causal relationship is questionable because the possibility of a reverse causal relationship (*ie*, thrombosis and other potential confounders result in increased MP formation) cannot be excluded in some instances.

Briefly, MPs are postulated to mediate several of the following aspects of cancer: the release of TF-bearing MPs derived from both cancer cells and host cells may lead specifically to a hypercoagulable state in cancer patients. MPs may contribute to malignancy propagation by promoting angiogenic processes, impairing both the immune response and cell engraftment, which may lead to an additional release of MPs. Additional studies are required to better clarify the relationships among MPs, cancer and VTE.

Therapeutic potential of MPs

We summarized several approaches by which therapeutic agents may counteract MPs prothrombotic actions and carcinogenesis based on past research. First, oncogene-directed treatments may have a profound impact on the morbidity and survival of oncologic patients by attenuating the prothrombotic effects of MPs in cancer[81]. Blocking MP-induced intercellular communication is another approach. P-selectin is a key endothelial cell receptor that captures circulating leukocytes and leukocyte-derived MPs expressing PSGL-1[82]. Emerging studies have suggested that blocking the binding of leukocytes and MPs to activated endothelium via P-selectin inhibitors may represent a novel strategy for reducing the

incidence of VTE[67–69, 83–87]. Inducing angiogenesis regulation is a third approach. MPs are involved in many complex activities associated with angiogenesis. Although the mechanisms underlying these activities remain unclear, considerable effort has been expended to use MPs as therapeutic tools in diseases characterized by altered angiogenesis[88]. MPs may also be used as vectors for the delivery of chemotherapeutic drugs. Because MPs apparently exhibit homing ability, they may be useful "cargo" for the targeted delivery of therapeutic agents because they possess properties that enable them to localize to specific areas of target issues. Several studies have proposed a role for MPs in atherosclerosis, transplantation and cancer[41, 89–91]. Although recent progress has been made in the therapeutic use of MPs, further research is necessary because we cannot blindly apply them as therapeutic agents without a complete and clear understanding of the mechanisms underlying both MP formation and activity.

Conclusions and perspectives

MPs play a pivotal role in the initiation and propagation of VTE through the development of their own procoagulant properties, enhancing intercellular communication and promoting inflammation. Additionally, MPs are involved in crosstalk between cancer and VTE. MPs may not only exert deleterious effects in the setting of VTE but may also act as novel diagnostic markers and provide new and exciting opportunities and therapeutic approaches used to deter VTE and attenuate the prothrombotic effects of cancer. Nevertheless, further prospective clinical studies are required to investigate those effects, and standardized assays with high rates of reproducibility must be developed to avoid discrepancies caused by the various methods of MP measurement currently in use.

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Abbreviations

CPMP, circulating procoagulant microparticle; DVT, deep venous thrombosis; EC, endothelial cell; EMP, endothelial microparticle; EMPTF, tissue factor-bearing microparticle derived from endothelial cells; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MP, microparticle, MPTF, tissue factor-bearing microparticle; MV, microvesicle; PE, pulmonary embolism; PLC, conjugates between platelets and leukocytes; PMP, platelet microparticle; PMPTF, tissue factor-bearing microparticle derived from platelets; PS, phosphatidylserine; PSGL-1, P-selectin glycoprotein ligand-1; TF, tissue factor; vWF, von Willebrand factor; VTE, venous thromboembolism.

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