Review

Biomarkers of Coagulation Disorders—Where to from Here?

Benjamin Reardon [1,2], Leonardo Pasalic [3,4,5] and Emmanuel J. Favaloro [3,4,6,7,*]

[1] Joint Medical Program, School of Medicine and Public Health, University of Newcastle, Callaghan, NSW 2145, Australia; benjamin.reardon@health.nsw.gov.au
[2] Haematology Department, Calvary Mater Hospital Newcastle, Waratah, NSW 2298, Australia
[3] Haematology Department, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead Hospital, Westmead, NSW 2145, Australia; leonardo.pasalic@health.nsw.gov.au
[4] Sydney Centres for Thrombosis and Haemostasis, Research and Education Network (REN) and ICPMR, Westmead Hospital, Westmead, NSW 2145, Australia
[5] Westmead Clinical School, University of Sydney, Westmead Hospital, Westmead, NSW 2145, Australia
[6] School of Dentistry and Medical Sciences, Faculty of Science and Health, Charles Sturt University, Wagga Wagga, NSW 2650, Australia
[7] School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Westmead Hospital, Westmead, NSW 2145, Australia
* Correspondence: emmanuel.favaloro@health.nsw.gov.au

Abstract

Disorders of thrombosis and bleeding contribute to a significant morbidity and mortality burden worldwide. Correctly identifying contributing factors towards either thrombosis or bleeding carries implications for diagnosis, prognosis and management. Although there are established and commonly used biomarkers for both circumstances, the complexity of hemostasis contributes to the wide variability in assay methodology and information provided by each individual assay. There are several emerging biomarkers of thrombosis and hemostasis, which require further evaluation of their roles in diagnosis and management in specific patient populations. This narrative review summarizes established, emerging, and exploratory biomarkers of both thrombosis and bleeding disorders, outlining their biological roles, diagnostic utility, and limitations, with a particular focus on clinical relevance, assay methodology and future directions.

Keywords: biomarker; coagulation; bleeding; thrombosis; thrombophilia

1. Introduction

Biomarkers are measurable indicators of biological processes, disease states or responses to therapeutic interventions [1]. Biomarkers of coagulation thus serve as markers of a prothrombotic state or increased bleeding tendency, providing insights into the temporal dynamics of thrombogenesis and potential therapeutic targets [2]. Venous thromboembolism (VTE) and cardiovascular disorders, including acute myocardial infarction and ischemic stroke, are leading contributors to long-term morbidity and mortality with shared common risk factors [3]. The clinical relevance of biomarkers in coagulation is therefore very high.

VTE, including deep vein thrombosis (DVT) and pulmonary embolism (PE), is currently the third leading cause of vascular-related mortality following myocardial infarction and stroke [4,5], where prompt and accurate diagnosis is critical yet challenging due to variable clinical presentations. Combinations of biomarkers of thrombosis, both venous and arterial, may aid in diagnosis, risk stratification and management in the acute care

Academic Editor: Felice Amato

Received: 11 January 2026
Revised: 23 January 2026
Accepted: 26 January 2026
Published: 3 February 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

setting. We aim to describe the commonly used biomarkers of bleeding and thrombosis, as well as those that have a growing evidence base but are not widely available or well known.

2. Established Biomarkers in Clinical Practice

2.1. Standard Coagulation Testing

Blood clotting studies remain a crucial component in the initial assessment of a patient's coagulation status. Core coagulation evaluation encompasses readily available tests, such as prothrombin time (PT)/international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen, platelet count, D-dimer and sometimes fibrin/fibrinogen degradation products. These tests provide basic but valuable insight into a patient's risk of bleeding or thrombosis. These tests are not without their substantial limitations. Coagulation is a complex and dynamic process involving multiple pathways, and in vitro testing may not reflect the in vivo processes [6]. Furthermore, the pre-analytical aspects of testing need to be considered for their correct interpretation [7].

2.2. APTT and PT/INR

The APTT and PT measure time to clot formation in intrinsic and extrinsic coagulation pathways, respectively [8]. Prolongation of either may be secondary to one or more factor deficiencies, drug effects or the presence of an inhibitor. Normal PT values range from around 9 to 13 s, and prolongation suggests potential reductions in extrinsic and common pathway clotting factors (V, VII, X and prothrombin [II]), anticoagulant effect (such as apixaban, rivaroxaban or warfarin), liver disease or vitamin K deficiency [9]. Systemic inflammation can lead to an overexpression of tissue factors on activated endothelial cells and monocytes, promoting coagulation and thrombin generation [10]. The INR is a mathematical formula that aims to help standardize PT values between laboratories and is primarily used for warfarin (or vitamin K antagonist; VKA) monitoring. The INR is represented by $INR = (PT/MNPT)^{ISI}$, where the PT is the patient's PT, typically derived from an automated coagulation analyzer, the MNPT is the mean normal PT of at least 20 normal individuals using the same method as the patient's PT, and the ISI is the international sensitivity index for the PT reagent/instrument in use [11]. The ISI is usually provided by the PT reagent manufacturer.

Normal APTT values range from around 25 to 35 s, and prolongation suggests potential reduction in intrinsic pathway (VIII, IX, XI, XII) and common pathway (II, V, X) clotting factors, the presence of an inhibitor (including lupus anticoagulant), anticoagulant effect (such as apixaban, rivaroxaban and heparin) and possibly liver disease (although the effect may be lower for APTT than for PT, since factor VIII is primarily produced by endothelial cells) [6,9].

Prolongation of both APTT and PT has been described as a biomarker of trauma-induced coagulopathy [12]. Alternatively, shortening of APTT and PT can be seen in some patients; for APTT, this may be due to elevated FVIII levels and is a risk factor for thrombosis [13]. Alternatively, pre-analytical issues may yield both shortening or prolongation of PT and APTT and require exclusion prior to the release of test results.

2.3. Fibrinogen

Fibrinogen testing is generally performed as a functional assay based on the time to clot formation following the addition of an excess of thrombin. Fibrinogen is an acute-phase reactant and is elevated in states of inflammation, but is reduced in consumptive coagulopathy and liver disease. Fibrinogen has been reported as a biomarker of disseminated intravascular coagulation (DIC) [14,15], trauma-induced coagulopathy [12] and coagulopathy of sepsis [10]. Hypofibrinogenemia or dysfibrinogenemia may reflect rare congenital

disorders of bleeding with a highly heterogeneous phenotype [16]. Alternate acquired deficiencies of fibrinogen are also associated with bleeding [6]. In contrast, elevations in fibrinogen may be associated with thrombosis [2].

2.4. Platelet Count

A reduction or increase in platelet counts in patients has many causes [17], and its use as a biomarker of thrombosis or bleeding has shown varying specificity. Severe thrombocytopenia (<10–20,000/µL of blood) has a bleeding risk, which is typically mitigated with platelet transfusion or immunosuppression for immune-mediated causes. Inherited causes of thrombocytopenia should also be considered, but platelet count and blood film assessment are inadequate for a definitive diagnosis. Primary causes of platelet count elevation (>450,000/µL of blood) include myeloproliferative neoplasms and are associated with an increased risk of thrombosis as well as bleeding compared to secondary thrombocytosis [18,19]. Secondary thrombocytosis can be due to iron deficiency, infection, post-splenectomy, inflammatory conditions, malignancy (non-myeloproliferative disorders) or exercise and is rarely associated with thrombotic events, including myocardial infarction or VTE [20].

2.5. D-Dimer and Fibrin/Fibrinogen Degradation Products

Fibrin/fibrinogen degradation products (FDPs), including D-dimer, are fragments generated during the enzymatic breakdown of fibrinogen and fibrin by plasmin [21]. D-dimer is a fibrin degradation product derived specifically from clot breakdown, and therefore, is more predictable of thrombosis than other FDPs [22]. Normal D-dimer values are typically less than 500 ng/mL in fibrinogen equivalent units (FEU), but high variability in assay sensitivity and specificity of D-dimer and other FDPs can impact their interpretation [23]. D-dimer is elevated in VTE and DIC [24]. Notably, D-dimer and FDPs in general are not specific to VTE and can be elevated in other hypercoagulable states, including pregnancy, trauma, and malignancy [25–28].

2.6. Thrombophilia Testing

Thrombophilia defines a condition in which there is an increased risk of thrombosis, usually VTE, but may also include arterial thrombosis [29]. Table 1 summarizes the key components of thrombophilia testing, including phenotypic and genetic tests associated with an increased risk of thrombosis. These assays are used as biomarkers of thrombotic disorders. In addition, increases in coagulation factor levels can also reflect thrombosis risk [2].

Table 1. Summary of biomarkers of thrombophilia.

Test	Abbreviation	Purpose	Type of Assay
Activated protein C resistance	APCR	'Resistance' to activated protein C inactivation of factors Va and VIIIa. People with APCR are at high risk of VTE.	Several different versions, with some based on aPTT and others based on snake venom activation of coagulation [30,31]. Most cases of APCR are due to genetic causes such as factor V Leiden (FVL). However, depending on the assay, laboratory-detected APCR can also reflect other conditions such as elevated levels of factor VIII and pregnancy-induced hemostasis changes.

Table 1. *Cont.*

Test	Abbreviation	Purpose	Type of Assay
Antithrombin	AT	The level ('antigen') or activity (functional assays) of AT. AT is a serine protease inhibitor, or SERPIN, that operates as a natural anticoagulant to inactivate predominantly thrombin (FIIa) and FXa, and to a lesser extent, the other active enzymes of coagulation biochemistry. Deficiency of AT can lead to thrombophilia, primarily VTE.	AT activity assays are most common and measure the level of functionally active AT through inhibition of FIIa or FXa. Antigen-based assays may be useful to supplement activity assays and help characterize AT defects. *SERPINC1* gene mutation testing: Next-generation sequencing (NGS) or PCR-based testing can be used to distinguish congenital or acquired deficiency.
Protein C	PC	The level ('antigen') or activity (functional assays) of PC. PC is the vitamin K-dependent zymogen of the serine protease-activated protein C (APC), which serves a critical role in the regulation of thrombin generation by inactivating FVa and FVIIIa, the cofactors for the prothrombinase and tenase complexes, respectively. Deficiency of PC can lead to thrombophilia, primarily VTE.	PC activity assays are most common and measure the level of functionally active PC in chromogenic or clotting assays. Antigen-based assays may be useful to supplement activity assays and help characterize PC defects. *PROC* gene mutation: NGS or PCR-based testing can be used to distinguish congenital or acquired deficiency.
Protein S	PS	The level ('antigen') or activity (functional assays) of PS. PS is a vitamin K-dependent nonenzymatic cofactor for both APC and tissue factor pathway inhibitor (TFPI), thereby contributing to the downregulation of coagulation in both the initiation and propagation phases. Deficiency of PS can lead to thrombophilia, primarily VTE.	Antigen-based assays for 'Free' PS (i.e., not bound to C4b-binding protein (C4BP)) are most commonly performed, as this form of PS reflects the functionally available form of PS. Some centers also evaluate 'total' PS levels by antigenic assays. Clot-based PS assays are available but not generally recommended. *PROS1* gene mutation: NGS or PCR-based testing can be used to distinguish congenital or acquired deficiency.
Factor V Leiden mutation	FVL	Factor V Leiden mutation represents the most common congenital thrombophilia, affecting 3–8% of the Caucasian population [32]. FVL is a common genetic mutation of FV arising as a single nucleotide polymorphism that abolishes the predominant APC cleavage site in FVa, Arg506 (p. Arg534Gln).	*FV* gene PCR-based assay. Most methods are specific for FVL and may miss less common mutations of the *FV* gene associated with thrombosis. In these circumstances, Sangar sequencing or NGS may be utilized.
Prothrombin gene mutation	PGM	The prothrombin gene mutation G20210A occurs in approximately 2–3% of the general population, leading to elevated levels of prothrombin (Factor II), with an increased risk of venous thromboembolism [33].	Genetic testing of *F2* G2021A is typically performed by PCR assays. Most methods are specific for *F2* G20210A and may miss less common *F2* variants associated with thrombosis. In these circumstances, Sangar sequencing or NGS may be utilized.

https://doi.org/10.3390/biom16020235

Table 1. *Cont.*

Test	Abbreviation	Purpose	Type of Assay
Lupus anticoagulant	LA	Laboratory criteria for antiphospholipid syndrome (APS) associated with venous or arterial thrombosis, or obstetric criteria	At least 2 tests based on different assay principles for phospholipid-dependent prolongation of clotting: activated partial thromboplastin time (APTT) and dilute Russell viper venom time (dRVVT)-based. Of note, there are many factors which can affect the reliability of these assays and thus their interpretation [34].
anti–beta2-glycoprotein I and anticardiolipin antibodies	aβ2GPI and aCL	Laboratory criteria for APS associated with venous or arterial thrombosis, or obstetric criteria	Immunological assays and the presence of either immunoglobulin (Ig)G or IgM for aCL and/or aβ2GPI, confirmed after at least 12 weeks.
JAK2 V617F mutation	JAK2	*JAK2* V617F constitutes a point mutation localized within the Janus kinase 2. Mutations in *JAK2* lead to cellular proliferation and cytokine release, consistently observed in polycythemia vera and other myeloproliferative neoplasms, as well as increased thrombosis risk secondary to platelet activation, leukocytosis and altered endothelial function [35,36]. Hepatic and splanchnic vein thrombosis have a strong association with *JAK2* mutations [37].	PCR and ddPCR are most commonly used but may miss non-canonical mutations. Sangar sequencing or next-generation sequencing methods may be used for non-*JAK2* V617F mutations.
Paroxysmal nocturnal hemoglobinuria	PNH	PNH is characterized by uncontrolled complement activation leading to platelet activation and intravascular hemolysis [38]. Additionally, 29–44% of patients with PNH experience thrombosis, and thrombotic events are associated with high mortality in this population [39].	Flow cytometric-based testing on peripheral blood for detection of loss of GPI-anchored proteins across red cells, monocytes and granulocytes [40]. *PIG-A* mutations are not commonly tested, given the high reliability and availability of flow cytometry-based assays.

Largely adapted from information provided in Favaloro et al. (2025) [29]. Additional material from the table cited references.

2.7. Markers Normally Associated with Bleeding

Bleeding disorders are highly heterogeneous and vary significantly in bleeding phenotype and severity. Table 2 summarizes the key biomarkers of suspected bleeding disorders. It should be noted that fibrinogen testing is included in this table but has a highly variable clinical phenotype and may be associated with thrombosis [16]. Similarly, while deficiencies in coagulation factors and von Willebrand factor (VWF) are associated with bleeding, high levels of some factors (e.g., FVIII, FIX, VWF) are instead associated with thrombosis [2]. Although vitamin C deficiency and connective disorders have not been listed in the table below, they are underrecognized causes of excessive bleeding, particularly in patients with otherwise unremarkable screening tests of coagulation [41].

https://doi.org/10.3390/biom16020235

Table 2. Summary of biomarkers of bleeding disorders.

Test	Abbreviation	Purpose	Type of Assay
Factor levels	Factor II, V, VII, VIII, IX, X, XI, XII	Detection of factor deficiency (diagnostic of hemophilia A or B when factor VIII or IX is <40%, respectively) Differentials for low FVIII may include VWD or acquired VWS. Notably, FXII deficiency is not associated with a bleeding disorder, but may explain a prolonged APTT	1-stage clot-based or 2-stage chromogenic assay. Bovine-based chromogenic assays should be used in patients on emicizumab to measure residual factor VIII levels, including recombinant factor VIII [42]. Genetic testing of respective genes by PCR or NGS platforms.
Inhibitor Detection	Bethesda/Nijmegen	Detection of factor-specific inhibitor	Usually a clot-based assay with a mix of diluted patient plasma with pooled normal plasma, with Nijmegen modification for comparison of factor-deficient plasma [43]. Chromogenic-based assays used in some centers.
VWF studies	VWF:Ag, VWF:RCo, VWF:GPIbM, VWF:GPIbR, VWF:Ab, VWF:CB, VWF:FVIIIB	Detection of quantitative and qualitative VWF	Various assays with the use of assay ratios. Testing performed by ELISA, LIA, CLIA or flow cytometric methods [44].
VWF Multimer studies	VWF:Mult	Detection of loss of high- and sometimes intermediate-molecular-weight multimers in type 2A and 2B VWD	Gel electrophoresis.
Fibrinogen	Fibrinogen	Detection of hypofibrinogenemia or dysfibrinogenemia	Activity-based assay (Clauss) or antigen-based assay (LIA, EID or ELISA) Genotypes of *FGA*, *FGB* and *FGG* genes [45,46].
Platelet function analyzer studies	PFA-100 or PFA-200	Main benefit as a screening test for defects of primary hemostasis, especially VWD. Can detect many abnormalities in platelet function but lacks specificity	High shear rates through an aperture coated with collagen and epinephrine or collagen and ADP (PFA-100 and PFA-200) or prostaglandin E1 (PFA-200) to induce platelet adhesion and activation [47,48].
Platelet Aggregometry	LTA, WBA, Multiplate	Most commonly light transmission aggregometry (or whole blood (impedance) aggregometry), used for specificity of platelet defect from specific agonists (e.g., ADP, collagen, ristocetin, adrenaline, arachidonic acid, thrombin, thromboxane A2)	Tests platelet aggregation to particular agonists in vitro with detection of primary and secondary waves, generally using platelet rich plasma (LTA), although whole blood aggregometry (WBA) can be used [49].
Factor XIII	FXIII	Detection of FXIII deficiency	Clot solubility, immunological, functional (including ammonia release), or chromogenic assays [50].

https://doi.org/10.3390/biom16020235

Table 2. *Cont.*

Test	Abbreviation	Purpose	Type of Assay
Flow cytometry	FC	Used to detect the absence of CD41, CD61 and CD42b expression on platelets in Glanzmann thrombasthenia and Bernard Soulier syndrome	Labeling of platelets with antibodies directed against surface glycoproteins.
Blood Group	ABO	Detection of type O blood group, which is associated with lower VWF levels and activity	Forward blood group with anti-A, anti-B and anti-A,B.

Abbreviations: LTA, light transmission aggregometry; VWF, von Willebrand factor; VWF:Ab, VWF measured using a monoclonal antibody-based assay; VWF:Ag, VWF antigen; VWF:CB, VWF collagen binding; VWF:FVIIIB, VWF factor VIII binding; VWF:GPIbM, VWF (platelet) recombinant mutated glycoprotein Ib binding; VWF:GPIbR, VWF (platelet) recombinant glycoprotein Ib binding; VWF:RCo, VWF ristocetin cofactor.

3. Emerging Biomarkers

3.1. Endothelial Activation (Soluble Thrombomodulin)

Thrombomodulin is found on the endothelial surface and plays several roles in hemostasis. Thrombomodulin binds to thrombin, which can then activate protein C and the thrombin-activatable fibrinolysis inhibitor, ultimately attenuating fibrinolysis [51]. Soluble thrombomodulin levels are elevated following injury and have been associated with inferior clinical outcomes in patients following trauma [52–54]. An inherited bleeding condition from mutations in the thrombomodulin gene, *THBD*, has been reported to be associated with excessive bleeding following injury, reduced thrombin generation and slower rates of fibrinolysis [55–58]. Soluble thrombomodulin has also been found to be increased in patients following severe head and neck or extracranial trauma as a biomarker of trauma severity and its ensuing coagulopathy [59].

3.2. Thrombin–Antithrombin Complex (TAT)

Thrombin has pleiotropic effects on cellular processes and is canonical in coagulation, converting fibrinogen to fibrin and activating platelets via protease-activated receptors [12,59–61]. Thrombin has effects on cellular processes, including vascular permeability, promotion of oxidative stress and upregulation of pro-inflammatory cytokines [62]. Following thrombin formation, the natural anticoagulant antithrombin irreversibly binds to thrombin, leading to thrombin–antithrombin (TAT) complex formation, a validated marker of thrombin generation, particularly in the early stages of coagulation activation [60]. TAT complexes are more easily detected than thrombin [63]. Elevated TAT levels have been associated with coronary slow flow and proposed as a diagnostic tool [64], as well as in patients following myocardial infarction [65]. Elevated TAT levels have also been found in ischemic stroke, with higher levels closely associated with large-volume ischemic stroke, cardioembolic stroke and poor revascularization following stroke, as compared with atherothrombotic or lacunar strokes [63]. Elevated TAT levels have also been found in sepsis-associated coagulopathy [66], following intracranial hemorrhage as a poor prognostic marker, and have higher sensitivity and specificity than D-dimer in detecting VTE post-operatively [67]. TAT detection in the laboratory has some limitations, notably that its short half-life, susceptibility to proteolytic degradation, short time window for detection of elevation following VTE, and notably, some genetic mutations, like those of FVL, may affect TAT levels [68–70].

TAT and D-dimer testing has also been studied in patients with hemophilia A and B, following thrombosis after factor VIII or IX administration with marked elevated levels associated with intravascular thrombosis [70–72].

3.3. Platelet Activation and Adhesion (CD40L, P-Selectin)

Platelets are a key component in thrombosis and thrombotic diseases, where activated platelets mediate thrombus formation and are involved in multiple interactions with vascular cells, inflammatory cytokines, and the coagulation system [73]. Following activation, platelets release over 300 proteins, including P-selectin and CD40L, which are two potential biomarkers of platelet activation.

The CD40 ligand (CD40L) is a transmembrane molecule expressed in T-lymphocytes and platelets [74,75]. Interactions with CD40L are required for many immune responses; CD40L-induced CD40 signaling in B-cells from T-dependent antigens induces BCL6 and Ki67 expression in germinal center B cells, ultimately allowing Ig class switching [74]. The CD40-CD40L system is associated with both prothrombotic and pro-inflammatory effects.

Soluble CD40 is predominantly generated by platelets and contributes to the pathophysiology of atherosclerosis [75,76]. Soluble CD40L enhances platelet activation, aggregation, and platelet–leukocyte conjugation, which contributes towards atherothrombosis [75–78]. The action and interaction of CD40 and CD40L on endothelial cells is multifaceted and involves upregulation of adhesion molecules and pro-inflammatory cytokines cells [79], whilst also stimulating the expression of tissue factors by monocytes and endothelial cells [80] and contributing to endothelial dysfunction by decreasing nitric oxide (NO) synthesis and augmenting oxidative stress [81]. CD40 is also implicated as a key mediator in platelet-associated transfusion reactions [82,83]. Incubation of platelets with recombinant sCD40L can lead to enhanced P-selectin expression, platelet aggregation, and platelet–leukocyte conjugation [84].

P-selectin is another transmembrane glycoprotein embedded in alpha-granules and stored within Weibel–Palade bodies of vascular endothelial cells [73]. Upon platelet activation, the membrane of alpha-granules merges with the platelet membrane, leading to P-selectin translocation to the platelet surface and eventual cleaving and release of soluble P-selectin [85]. Soluble p-selectin (sP-selectin) is increased in patients with coronary artery disease and shown to be a reliable biomarker across different ethnicities of patients on antiplatelet therapies [86]. sP-selectin is elevated in patients with DVT and PE compared with healthy controls [87,88]. In patients with peripheral vascular disease who require stenting, elevated sP-selectin levels were associated with restenosis, and CD40L was not [89]. Reidl et al. looked at sP-selectin and CD40L, as well as other biomarkers, in cancer patients with VTE, cancer patients without VTE and healthy subjects, and found that sP-selectin was elevated in patients with VTE irrespective of cancer status, which was not found in CD40L and other biomarkers [90].

3.4. NETs

Neutrophil extracellular traps (NETs) are associated with the development of thrombosis [91]. As a unique form of cell death resulting in the release of condensed chromatin and granular proteins, NET formation and translocation of reactive oxygen species, myeloperoxidase, histones and neutrophil elastase, they form a scaffold in which platelets, fibrinogen and VWF can adhere and aggregate [92]. Extracellular DNA staining positive for myeloperoxidase, histones and neutrophil elastase has been seen in immunostaining of venous thrombosis [93]. In baboons with iliac vein thrombosis, dotted and diffuse staining of DNA and positive staining of DNA–histone could be seen, which has also been seen in human venous thrombi from surgical samples [92,94]. The role of NETs as a biomarker in venous thromboembolism has been evaluated in a number of studies, including the use of plasma DNA levels, neutrophil elastase and histones (H3Cit-DNA), all of which were elevated in patients with VTE [95,96]. In subsequent studies of the use of plasma DNA and nucleosomes, NET biomarkers were higher in elderly patients with

https://doi.org/10.3390/biom16020235

PE compared to those with DVT [97], and subsequently, higher levels of plasma DNA and calprotectin were observed in patients with splanchnic vein thrombosis compared with DVT [98]. NET biomarkers (DNA, myeloperoxidase, nucleosomes) have also been used to predict severity, clinical outcome and hypercoagulability in patients with coronary artery disease and acute myocardial infarction [99–103]. Myeloperoxidase–DNA and histone (H3Cit) have also been shown to be higher in patients with COVID-19 infection compared with healthy controls, with levels correlating with disease severity [104,105]. A number of studies related to the use of NET-based targets are entering the clinical space, particularly in degrading NET formation by use of DNase [106], heparin in its removal of histones in chromatin [92], and JAK1/JAK2 inhibition by abrogating NET formation and reduction in stenosis-induced venous thrombosis [107]. Neutrophil elastase produced by NETs may also be implicated in suppressed fibrinolysis, which may promote persistent microvascular thrombosis and organ dysfunction in critically unwell patients [108].

3.5. Extracellular Vesicles

Extracellular vesicles (EVs) are a heterogeneous group of vesicle-derived particles, including phospholipids, tissue factor, P-selectin and other procoagulant proteins, which are released to the extracellular environment in response to inflammatory or proapoptotic factors [109]. EVs are released following a number of stimuli, including IL1α, IL1β, LPS, PMA, TNF α, complement C5b-0, PAI-1, thrombin, staurosporine, ADP, and collagen [110–114]. Many of these stimuli are prothrombotic for EV release; however, they may exert prothrombotic activity themselves [109]. EVs have both direct and indirect effects on platelets, white cells, red cells and coagulation factors [109]. EVs isolated from healthy participants were found to support coagulation in vitro via TF-independent pathways [115]. Measurement of fibrin generation as a surrogate for EV–tissue factor generation has been demonstrated in cancer patients with VTE, with higher fibrin generation being associated with a two-fold increased risk of VTE (HR 2.0, 95% CI 1.1–3.6) [116]. More recently, EVs were associated with elevated interleukin-1B and thrombosis related to antiphospholipid syndrome [117]. In a prospective study by Antic et al. (2023), 127 patients diagnosed with lymphoma who developed VTE events had higher tissue factor values (224.41 [93.1–446.0] (median [IQR]) vs. 136.43 [109.4–193.4], p = 0.036) compared with patients who did not develop VTE, and patients who had aggressive lymphoma had an 8-fold increase in CD11b+/CD16+ neutrophils, a 2-fold increase in CD61+ platelets and a 3-fold increase in CD31+ endothelial cells labeled with TF and extracellular vesicles. Interestingly, P-selectin levels were not found to be different between groups [118].

3.6. Plasmin–Alpha2-Antiplasmin Complex (PAP)

Plasmin and α2-antiplasmin (α2-AP) are key regulators of fibrin polymer dissolution into soluble fragments. Free plasmin rapidly combines with α2-AP to form PAP [119]. Elevated PAP levels indicate active thrombolysis and thrombus formation [120–122]. PAP more directly reflects plasma plasmin activity and rises earlier than D-dimer in acute VTE [123]. Ma et al. (2025) found that in patients with gynecological malignancy undergoing surgery, PAP area under the receiver operating curve (ROC) was 0.95 preoperatively and 0.941 postoperatively in patients who developed VTE, indicating its use as a predictive biomarker of VTE [123]. However, in another study by Folsom et al. (2003) looking at 308 patients with VTE and 640 controls, there was no significant association of fibrinolytic variables, including PAP, after adjusting for other risk factors and patient age [124].

3.7. Epigenetic and Genetic Testing

VTE and arterial thrombotic events are polygenic in nature; however, it is increasingly acknowledged that environmental factors and histone modification contribute towards

genomic expression and inheritability [3]. MicroRNAs, long non-coding RNAs and extracellular vesicles have been identified as key elements in regulating gene expression in coagulation [125]. A systematic review by Patsouras and Vlachoyiannopoulos (2019) [126] identified two independent studies examining genome-wide methylation profiles in patients with cardiovascular disease, incorporating a total of 1080 cases, and found that differential DNA methylation in CpG sites in genes is implicated in cardiac function, cardiovascular disease and response to ischemic injury [127,128]. The association of systemic autoimmune disease and cardiovascular disease has been well established [129]. In DNA isolated from patients with rheumatoid arthritis and Kawasaki disease, methylation differences have been seen in pathways involving coagulation and platelet activation, including protein C inhibitor, P-selectin, ICAM1, VWF and MAPK14 genes [130,131].

Epigenetic factors contributing towards thrombosis have also been found in APS, with a significant reduction in methylation in the *IL8* promoter gene and an increased tissue factor compared with healthy controls [126]. Neutrophils isolated from patients with APS and SLE compared with healthy controls have demonstrated 42 differentially methylated CpG sites, which may also contribute to thrombosis [132].

Epigenetics have also been identified as a contributor to cancer-associated thrombosis. Cuff et al. found that hypomethylation of the promoter of the transcription factor of genes associated with coagulation in patients with renal or ovarian clear cell carcinoma was associated with a 2.3-fold increased risk of clinically significant venous thrombosis [133]. Factor X and tissue factor promoter methylation levels were found to be lower in glioma specimens, which correlated with intra-tumor microthrombi and VTE [134,135]. In patients with primary myelofibrosis, methylation of CD18 has been found to be higher than in healthy controls and found to be an independent prognostic factor of thrombosis [136]. This is particularly of note given the interaction of CD11b and CD18 as a leukocyte-specific integrin receptor promoting platelet activation, neutrophil recruitment and endothelial injury [137].

Given the plethora of tests required for the investigation of potential inherited bleeding disorders, genetic testing is being increasingly used to define pathogenic variants and allow for the ultimate classification of disease and its severity [138]. Next-generation sequencing (NGS) allows the ability to test multiple genes of interest (panel-based) and may allow for the discovery of variants previously not known or unclassified (whole exome or whole genome sequencing) [139]. Genetic testing may also assume a key role in bleeding conditions with mild phenotypes that remain unresolved with functional or protein-based testing [140,141]. Genetic testing may also have high clinical utility in the detection of inherited platelet disorders, given that the diagnosis is often more challenging due to high clinical heterogeneity and indistinct laboratory features [142–144]. Pezeshkpoor et al. (2022) propose genetic testing as the final definitive test required for precise classification and allowing for greater prediction of clinical bleeding risk [145]. Current trials are underway, investigating the use of early genetic screening in suspected inherited bleeding disorders [146].

3.8. Viscoelastic Testing

Viscoelastic testing (VET) is increasingly being used in clinical and research settings to assess hemostasis [6,147]. Whilst there are a number of pre-test clinical scores to assess the risk of hemostasis dysfunction, their reliability and applicability in different patient populations is unclear [148]. A number of studies have used VET to assess hypercoagulability and patients at increased risk of thromboembolism. Maximum clot strength (or maximum amplitude, MA) is the most common component of VET used to assess these conditions. In a retrospective cohort study on hospitalized patients over the age of 65, Zheng et al. (2014)

https://doi.org/10.3390/biom16020235

found that the prevalence of venous or arterial thrombotic events was 20.6% in patients with an MA value of >69 mm on VET [149]. In a prospective cohort study on critically ill adult patients, Tartamella et al. (2016) found that the use of a thrombodynamic ratio (MA multiplied by the alpha angle) of >10.6 was associated with a 10.6% rate of VTE [150]. VET parameters have also been shown to reliably detect the hypercoagulable state peripartum and postpartum [151].

Viscoelastic testing is also being researched in its detection of bleeding states, including in patients on direct oral anticoagulants [152], liver disease [153], orthotopic liver transplantation [154], cardiac surgery [155], trauma [156], obstetric-related bleeding [157] and pediatrics [6,147,158]; however, there is a paucity in randomized or prospective studies in some of these populations [147].

3.9. Interleukin-6 (IL-6) and Interleukin-7 (IL-7)

The use of additional biomarkers may also be useful in patients with hemophilia experiencing acute bleeding. In a study by Knowles et al. (2023), recent bleeding events within 1 month, as well as acute bleeding presentations, correlated with elevated IL-6 levels in patients with hemophilia A and B, compared with non-bleeding patients with hemophilia [159].

Hemophilia-associated arthropathy is a significant cause of morbidity in patients with limited biomarker testing. In a comparison of rheumatoid- and hemophilia-associated arthropathy, Toenges et al. assessed 42 parameters in 129 male patients, including cytokines, angiogenesis and acute-phase reactants, to better establish a biomarker of arthropathy [160]. When comparing patients with rheumatoid arthritis to patients with hemophilia without associated arthropathy, IL-7 levels were increased non-significantly ($p = 0.24$), and VEGR-1 was decreased ($p = 0.002$); and in hemophilia patients with arthropathy, only serum ferritin was decreased ($p = 0.008$), compared with patients with rheumatoid arthritis.

4. Biomarker Validation and Translational Challenges

4.1. Regulatory Considerations

Regulators increasingly require a context of use (COU)-anchored framework in the establishment of new biomarkers, allowing for reliability in its specific interpretation and application in a clinical context [161]. Assay development requires testing of precision, accuracy, linearity, selectivity, stability and traceability of biomarkers measured, a large undertaking that requires extensive investment of time and resources [162]. In the development of many of the above biomarkers, testing will also need to include prospective studies and how management (including anticoagulant therapy or factor replacement therapy) affects the assay to avoid verification bias and demonstrate real-world prognostic value [163]. Laboratory capacity constraints may also influence assay uptake and rollout [164].

4.2. Point-of-Care Testing

Point-of-care (POC) testing comprises a unique challenge to assay development with specific ISO requirements for implementation [165]. POC testing offers the benefit of rapid turnaround time but is often competing with central laboratory assays which usually have better precision and lower bias [166]. Thus, POC testing head-to-head evaluations with central laboratory testing usually offer acceptable but imperfect agreement due to device-specific reagents, drug effects and calibration affecting universal intervals and clinical cut-offs [167,168].

https://doi.org/10.3390/biom16020235

4.3. Integration into Clinical Practice

The process leading to the eventual use of a biomarker in clinical practice can often span decades and can involve international collaboration between laboratories, scientists and clinicians. Vasan (2006) outlines a step-wise approach to biomarker development involving laboratory-based discovery, assay standardization, the establishment of reference intervals among healthy and diseased individuals, validation, and ultimately, regulatory approval for clinical use [2,169].

Multimodal biomarkers, including protein testing, cellular phenotype and genetic testing, may be utilized to better define clinical phenotypes of bleeding or thrombosis [170].

5. Conclusions and Future Directions

Biomarkers of coagulation and bleeding provide essential insights into thrombotic and hemorrhagic disorders, spanning established assays as well as comprehensive thrombophilia testing and evaluation of potential bleeding diathesis. Methodological heterogeneity remains a major barrier, where differing reagents, platforms, and interpretive criteria result in variable performance across laboratories. Emerging biomarkers—including soluble thrombomodulin, thrombin–antithrombin complexes, platelet activation markers, NET-derived products, extracellular vesicles, and fibrinolytic markers—offer a more dynamic assessment of coagulation activation and endothelial or inflammatory processes, though analytic variability and assay susceptibility to drug effects require further evaluation.

Future progress requires harmonization of assay methodology, formal establishment of reference intervals, and multicenter validation of emerging biomarkers within clearly defined clinical contexts of use. Integrating biomarker data into structured scoring systems may also strengthen precision when used diagnostically and allow for risk stratification for both thrombosis and bleeding, particularly where multimodal biomarker panels can be implemented. Expanding genetic and epigenetic testing, coupled with biomarker-based phenotyping, will likely refine disease classification and personalized treatment strategies, supporting more robust prediction of thrombotic and bleeding risk in complex or ambiguous cases.

Author Contributions: Conceptualization, all authors; writing—original draft preparation, B.R.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: As this manuscript represents a narrative review, or synthesis of existing literature, no new data were created.

Acknowledgments: This review represents the authors' viewpoints and may not reflect those of our associated institutions.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

VTE	Venous thromboembolism
PE	Pulmonary embolism
DVT	Deep venous thrombosis

PT	Prothrombin time
APTT	Activated partial thromboplastin time
INR	International normalized ratio
MNPT	Mean normal prothrombin time
DIC	Disseminated intravascular coagulation
FDPs	Fibrinogen degradation products
FEU	Fibrinogen equivalent units
TAT	Thrombin–antithrombin complex
CD40L	CD40 ligand
NET	Neutrophil extracellular trap
EV	Extracellular vesicle
PAP	Plasmin-alpha2-antiplasmin complex
POC	Point of care
NGS	Next-generation sequencing
VET	Viscoelastic testing
IL6	Interleukin-6
IL7	Interleukin-7
LTA	Light transmission aggregometry
VWF	von Willebrand factor
VWF:Ag	VWF antigen
VWF:CB	VWF collagen binding
VWF:FVIIIB	VWF factor VIII binding
VWF:GPIbM	VWF (platelet) recombinant mutated glycoprotein Ib binding
VWF:GPIbR	VWF (platelet) recombinant glycoprotein Ib binding
VWF:RCo	VWF ristocetin cofactor

References

1. Biomarkers Definitions Working Group. Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. *Clin. Pharmacol. Ther.* **2001**, *69*, 89–95. [CrossRef]
2. Shaw, J.R.; Nopp, S.; Stavik, B.; Youkhana, K.; Michels, A.L.; Kennes, S.; Rak, J.; Cate, H.T. Thrombosis, Translational Medicine, and Biomarker Research: Moving the Needle. *J. Am. Heart Assoc.* **2025**, *14*, e038782. [CrossRef]
3. Benincasa, G.; Costa, D.; Infante, T.; Lucchese, R.; Donatelli, F.; Napoli, C. Interplay Between Genetics and Epigenetics in Modulating the Risk of Venous Thromboembolism: A New Challenge for Personalized Therapy. *Thromb. Res.* **2019**, *177*, 145–153. [CrossRef]
4. Wendelboe, A.; Weitz, J.I. Global Health Burden of Venous Thromboembolism. *Arterioscler. Thromb. Vasc. Biol.* **2024**, *44*, 1007–1011. [CrossRef] [PubMed]
5. Yamashita, Y.; Morimoto, T.; Kadota, K.; Takase, T.; Hiramori, S.; Kim, K.; Oi, M.; Akao, M.; Kobayashi, Y.; Toyofuku, M.; et al. Causes of Long-Term Mortality in Patients with Venous Thromboembolism in the Real World: From the COMMAND VTE Registry. *Thromb. Res.* **2022**, *219*, 30–39. [CrossRef]
6. Reardon, B.; Pasalic, L.; Favaloro, E.J. The Role of Viscoelastic Testing in Assessing Hemostasis: A Challenge to Standard Laboratory Assays? *J. Clin. Med.* **2024**, *13*, 3612. [CrossRef] [PubMed]
7. Gosselin, R.C.; Marlar, R.A. Preanalytical Variables in Coagulation Testing: Setting the Stage for Accurate Results. *Semin. Thromb. Hemost.* **2019**, *45*, 433–448. [CrossRef] [PubMed]
8. Zaidi, S.R.H.; Rout, P. Interpretation of Blood Clotting Studies and Values (PT, PTT, aPTT, INR, Anti-Factor Xa, D-Dimer). In *StatPearls [Internet]*; StatPearls Publishing: Treasure Island, FL, USA, 2025. [PubMed]
9. Favaloro, E.J.; Pasalic, L.; Lippi, G. Getting Smart with Coagulation. *J. Thromb. Haemost.* **2022**, *20*, 1519–1522. [CrossRef]
10. Curtiaud, A.; Iba, T.; Angles-Cano, E.; Meziani, J.; Helms, J. Biomarkers of Sepsis-Induced Coagulopathy: Diagnostic Insights and Potential Therapeutic Implications. *Ann. Intensive Care* **2025**, *15*, 12. [CrossRef]
11. Favaloro, E.J.; Arunachalam, S.; Chapman, K.; Pasalic, L. Continued Harmonization of the International Normalized Ratio (INR) Across a Large Laboratory Network: Evidence of Sustained Low Inter-Laboratory Variation and Bias After a Change in Instrumentation. *Am. J. Clin. Pathol.* **2025**, *163*, 28–41. [CrossRef]
12. Genét, G.F.; Johansson, P.I.; Meyer, M.A.; Sølbeck, S.; Sørensen, A.M.; Larsen, C.F.; Welling, K.L.; Windeløv, N.A.; Rasmussen, L.S.; Ostrowski, S.R. Trauma-Induced Coagulopathy: Standard Coagulation Tests, Biomarkers of Coagulopathy, and Endothelial Damage in Patients with Traumatic Brain Injury. *J. Neurotrauma* **2013**, *30*, 301–306. [CrossRef]

13. Mina, A.; Favaloro, E.J.; Koutts, J. Relationship Between Short Activated Partial Thromboplastin Times, Thrombin Generation, Procoagulant Factors and Procoagulant Phospholipid Activity. *Blood Coagul. Fibrinolysis* **2012**, *23*, 203–207. [CrossRef] [PubMed]
14. Delabranche, X.; Quenot, J.P.; Lavigne, T.; Mercier, E.; François, B.; Severac, F.; Grunebaum, L.; Mehdi, M.; Zobairi, F.; Toti, F.; et al. Early Detection of Disseminated Intravascular Coagulation During Septic Shock: A Multicenter Prospective Study. *Crit. Care Med.* **2016**, *44*, e930–e939. [CrossRef] [PubMed]
15. Helms, J.; Iba, T.; Connors, J.M.; Gando, S.; Levi, M.; Meziani, F.; Levy, J.H. How to Manage Coagulopathies in Critically Ill Patients. *Intensive Care Med.* **2023**, *49*, 273–290. [CrossRef]
16. Simurda, T.; Asselta, R.; Zolkova, J.; Brunclikova, M.; Dobrotova, M.; Kolkova, Z.; Loderer, D.; Skornova, I.; Hudecek, J.; Lasabova, Z.; et al. Congenital Afibrinogenemia and Hypofibrinogenemia: Laboratory and Genetic Testing in Rare Bleeding Disorders with Life-Threatening Clinical Manifestations and Challenging Management. *Diagnostics* **2021**, *11*, 2140. [CrossRef]
17. Eslick, R.; Cutts, B.; Merriman, E.; McLintock, C.; McDonnell, N.; Shand, A.; Clarke, L.; Ng, S.; Kando, I.; Curnow, J. HOW Collaborative Position Paper on the Management of Thrombocytopenia in Pregnancy. *Aust. N. Z. J. Obstet. Gynaecol.* **2021**, *61*, 195–204. [CrossRef]
18. Schafer, A.I. Thrombocytosis. *JAMA* **2015**, *314*, 1171–1172. [CrossRef]
19. Rumi, E.; Cazzola, M. Diagnosis, Risk Stratification, and Response Evaluation in Classical Myeloproliferative Neoplasms. *Blood* **2017**, *129*, 680–692. [CrossRef] [PubMed]
20. Khan, P.N.; Nair, R.J.; Olivares, J.; Tingle, L.E.; Li, Z. Postsplenectomy Reactive Thrombocytosis. *Proc. (Bayl. Univ. Med. Cent.)* **2009**, *22*, 9–12. [CrossRef]
21. Chapin, J.C.; Hajjar, K.A. Fibrinolysis and the Control of Blood Coagulation. *Blood Rev.* **2015**, *29*, 17–24. [CrossRef]
22. Moresco, R.N.; Vargas, L.C.R.; Voegeli, C.F.; Santos, R.C.V. D-dimer and Its Relationship to Fibrinogen/Fibrin Degradation Products (FDPs) in Disorders Associated with Activation of Coagulation or Fibrinolytic Systems. *J. Clin. Lab. Anal.* **2003**, *17*, 77–79. [CrossRef] [PubMed]
23. Selby, R.; Meijer, P.; Favaloro, E.J. D-Dimer Diagnostics: Can I Use Any D-Dimer Assay? Bridging the Knowledge-to-Action Gap. *Res. Pract. Thromb. Haemost.* **2024**, *8*, 102335. [CrossRef] [PubMed]
24. Weitz, J.I.; Fredenburgh, J.C.; Eikelboom, J.W. A Test in Context: D-Dimer. *J. Am. Coll. Cardiol.* **2017**, *70*, 2411–2420. [CrossRef] [PubMed]
25. Kangro, K.; Wolberg, A.S.; Flick, M.J. Fibrinogen, Fibrin, and Fibrin Degradation Products in COVID-19. *Curr. Drug Targets* **2022**, *23*, 1593–1602. [CrossRef]
26. Rai, R.; Skbasu, B.; Jaiprakash, M. Fibrin and Fibrinogen Degradation Products in Malignancy. *Med. J. Armed Forces India* **2017**, *52*, 32–34. [CrossRef]
27. Hayakawa, M. Dynamics of Fibrinogen in Acute Phases of Trauma. *J. Intensive Care* **2017**, *5*, 3. [CrossRef]
28. Gaffney, P.J. Fibrin Degradation Products. *Ann. N. Y. Acad. Sci.* **2001**, *936*, 594–610. [CrossRef]
29. Favaloro, E.J.; Moore, G.W.; Pasalic, L. Diagnosing Thrombophilia: The Case for Genetic or Functional Testing? *Expert Rev. Mol. Diagn.* **2025**, *25*, 841–846. [CrossRef]
30. Moore, G.W. Thrombophilia Screening: Not So Straightforward. *Semin. Thromb. Hemost.* **2024**, *50*, 1131–1152. [CrossRef]
31. Bahraini, M.; Fazeli, A.; Dorgalaleh, A. Laboratory Diagnosis of Activated Protein C Resistance and Factor V Leiden. *Semin. Thromb. Hemost.* **2024**, *50*, 1067–1083. [CrossRef]
32. Bardan, C.R.; Ioniță, I.; Iordache, I.; Călămar-Popovici, D.; Todorescu, V.; Popescu, R.; Bernad, B.C.; Bardan, R.; Bernad, E.S. Epigenetic Biomarkers in Thrombophilia-Related Pregnancy Complications: Mechanisms, Diagnostic Potential, and Therapeutic Implications: A Narrative Review. *Int. J. Mol. Sci.* **2024**, *25*, 13634. [CrossRef]
33. Nguyen, A. Prothrombin G20210A Polymorphism and Thrombophilia. *Mayo Clin. Proc.* **2000**, *75*, 595–604. [CrossRef]
34. Favaloro, E.J.; Pasalic, L.; Selby, R. Testing for the Lupus Anticoagulant: The Good, the Bad, and the Ugly. *Res. Pract. Thromb. Haemost.* **2024**, *8*, 102385. [CrossRef]
35. Vannucchi, A.M.; Guglielmelli, P. JAK2 Mutation-Related Disease and Thrombosis. *Semin. Thromb. Hemost.* **2013**, *39*, 496–506. [CrossRef] [PubMed]
36. Horvat, I.; Boban, A.; Zadro, R.; Antolic, M.R.; Serventi-Seiwerth, R.; Roncevic, P.; Radman, I.; Sertic, D.; Vodanovic, M.; Pulanic, D.; et al. Influence of Blood Count, Cardiovascular Risks, Inherited Thrombophilia, and JAK2 V617F Burden Allele on Type of Thrombosis in Patients with Philadelphia Chromosome Negative Myeloproliferative Neoplasms. *Clin. Lymphoma Myeloma Leuk.* **2019**, *19*, 53–63. [CrossRef]
37. Kiladjian, J.J.; Cervantes, F.; Leebeek, F.W.G.; Marzac, C.; Cassinat, B.; Chevret, S.; Cazals-Hatem, D.; Plessier, A.; Garcia-Pagan, J.C.; Darwish Murad, S.; et al. The Impact of JAK2 and MPL Mutations on Diagnosis and Prognosis of Splanchnic Vein Thrombosis: A Report on 241 Cases. *Blood* **2008**, *111*, 4922–4929. [CrossRef] [PubMed]
38. Hill, A.; Kelly, R.J.; Hillmen, P. Thrombosis in Paroxysmal Nocturnal Hemoglobinuria. *Blood* **2013**, *121*, 4985–4996. [CrossRef] [PubMed]

39. de Latour, R.P.; Mary, J.Y.; Salanoubat, C.; Terriou, L.; Etienne, G.; Mohty, M.; Roth, S.; de Guibert, S.; Maury, S.; Cahn, J.Y.; et al. Paroxysmal Nocturnal Hemoglobinuria: Natural History of Disease Subcategories. *Blood* **2008**, *112*, 3099–3106. [CrossRef]
40. Australasian Cytometry Society. *ACS Guideline for Paroxysmal Nocturnal Haemoglobinuria Immunophenotyping*, 2nd ed.; reformatted to be read with ACS "Guidelines for Clinical Flow Cytometry Laboratory Practice"; Australasian Cytometry Society: Melbourne, Australia, 2017.
41. Leinøe, E.; Fridriksdottir, H.; Rasmussen, A.Ø.; Funding, E.; Sørensen, A.L.T.; Kampmann, P.; Lykkesfeldt, J.; Rossing, M. Low vitamin C status and hypermobility-related disorders in patients with bleeding disorder of unknown cause. *Haemophilia* **2024**, *30*, 1366–1372. [CrossRef]
42. Pfrepper, C.; Klamroth, R.; Ettingshausen, C.E.; Petros, S.; Siegemund, A.; Siegemund, T. Measurement of Recombinant Porcine Factor VIII in Patients with Congenital Haemophilia A and Inhibitors in the Presence of Emicizumab. *Haemophilia* **2024**, *30*, 545–553. [CrossRef]
43. Miller, C.H.; Platt, S.J.; Rice, A.S.; Kelly, F.; Soucie, J.M. Validation of Nijmegen-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. *J. Thromb. Haemost.* **2012**, *10*, 1055–1061. [CrossRef]
44. Favaloro, E.J.; Mohammed, S. Evaluation of a von Willebrand factor three test panel and chemiluminescent-based assay system for identification of, and therapy monitoring in, von Willebrand disease. *Thromb. Res.* **2016**, *141*, 202–211. [CrossRef] [PubMed]
45. Undas, A. Laboratory Testing for Fibrinogen Disorders: From Routine Investigations to Research Studies. *Semin. Thromb. Hemost.* **2025**, *51*, 651–659. [CrossRef]
46. Casini, A.; Moerloose, P.; Neerman-Arbez, M. Clinical, Laboratory, and Molecular Aspects of Congenital Fibrinogen Disorders. *Semin. Thromb. Hemost.* **2025**, *51*, 103–110. [CrossRef]
47. Favaloro, E.J. Description of an In Vitro Platelet Function Analyzer (PFA-100/PFA-200) 30 Years in the Making. *Semin. Thromb. Hemost.* **2024**, *50*, 320–324. [CrossRef] [PubMed]
48. Reny, J.L.; De Moerloose, P.; Dauzat, M.; Fontana, P. Use of the PFA-100 Closure Time to Predict Cardiovascular Events in Aspirin-Treated Cardiovascular Patients: A Systematic Review and Meta-Analysis. *J. Thromb. Haemost.* **2008**, *6*, 444–450. [CrossRef]
49. Gomez, K.; Anderson, J.; Baker, P.; Biss, T.; Jennings, I.; Lowe, G.; Platton, S.; British Society for Haematology Guidelines. Clinical and laboratory diagnosis of heritable platelet disorders in adults and children: A British Society for Haematology Guideline. *Br. J. Haematol.* **2021**, *195*, 46–72. [CrossRef]
50. Karimi, M.; Bereczky, Z.; Cohan, N.; Muszbek, L. Factor XIII Deficiency. *Semin. Thromb. Hemost.* **2009**, *35*, 426–438. [CrossRef] [PubMed]
51. Sillen, M.; DeClercq, P.J. Thrombin Activatable Fibrinolysis Inhibitor (TAFI): An Updated Narrative Review. *Int. J. Mol. Sci.* **2021**, *22*, 3670. [CrossRef]
52. Brohi, K.; Cohen, M.J.; Ganter, M.T.; Matthay, M.A.; Mackersie, R.C.; Pittet, J.-F. Acute Traumatic Coagulopathy: Initiated by Hypoperfusion, Modulated Through the Protein C Pathway? *Ann. Surg.* **2007**, *245*, 812–818. [CrossRef]
53. Johansson, P.I.; Stensballe, J.; Rasmussen, L.S.; Ostrowski, S.R. A High Admission Syndecan-1 Level, a Marker of Endothelial Glycocalyx Degradation, Is Associated with Inflammation, Protein C Depletion, Fibrinolysis, and Increased Mortality in Trauma Patients. *Ann. Surg.* **2011**, *254*, 194–200. [CrossRef]
54. Gando, S.; Wada, H.; Thachil, J. Differentiating DIC with the Fibrinolytic Phenotype from Coagulopathy of Trauma and Acute Coagulopathy of Trauma-Shock (COT/ACOTS). *J. Thromb. Haemost.* **2013**, *11*, 826–835. [CrossRef]
55. Westbury, S.K.; Whyte, C.S.; Stephens, J.; Downes, K.; Turro, E.; Claesen, K.; Mertens, J.C.; Hendriks, D.; Latif, A.L.; Leishman, E.J.; et al. A New Pedigree with Thrombomodulin-Associated Coagulopathy in Which Delayed Fibrinolysis Is Partially Attenuated by Co-Inherited TAFI Deficiency. *J. Thromb. Haemost.* **2020**, *18*, 2209–2214. [CrossRef]
56. Dargaud, Y.; Scoazec, J.Y.; Wielders, S.J.; Trzeciak, C.; Hackeng, T.M.; Négrier, C.; Hemker, H.C.; Lindhout, T.; Castoldi, E. Characterization of an Autosomal Dominant Bleeding Disorder Caused by a Thrombomodulin Mutation. *Blood* **2015**, *125*, 1497–1501. [CrossRef]
57. Osada, M.; Maruyama, K.; Kokame, K.; Denda, R.; Yamazaki, K.; Kunieda, H.; Hirao, M.; Madoiwa, S.; Okumura, N.; Murata, M.; et al. A Novel Homozygous Variant of the Thrombomodulin Gene Causes a Hereditary Bleeding Disorder. *Blood Adv.* **2021**, *5*, 3830–3838. [CrossRef]
58. Morrow, G.B.; Beavis, J.; Harper, S.; Bignell, P.; Laffan, M.A.; Curry, N. Characterisation of a Novel Thrombomodulin c.1487delC, p. (Pro496Argfs*10) Variant and Evaluation of Therapeutic Strategies to Manage the Rare Bleeding Phenotype. *Thromb. Res.* **2021**, *197*, 100–108. [CrossRef]
59. Aleksandrowicz, K.; Hempel, D.; Polityńska, B.; Wojtukiewicz, A.M.; Honn, K.V.; Tang, D.G.; Wojtukiewicz, M.Z. The Complex Role of Thrombin in Cancer and Metastasis: Focus on Interactions with the Immune System. *Semin Thromb Hemost.* **2024**, *50*, 462–473. [CrossRef] [PubMed]
60. Brodin, E.; Borvik, T.; Sandset, P.M.; Bonaa, K.H.; Nordoy, A.; Hansen, J.B. Coagulation Activation in Young Survivors of Myocardial Infarction—A Population-Based Case-Control Study. *Thromb. Haemost.* **2004**, *92*, 178–184. [CrossRef] [PubMed]

61. Crawley, J.T.; Zanardelli, S.; Chion, C.K.; Lane, D.A. The Central Role of Thrombin in Hemostasis. *J. Thromb. Haemost.* **2007**, *5*, 95–101. [CrossRef]
62. Borissoff, J.I.; Spronk, H.M.; Heeneman, S.; ten Cate, H. Is Thrombin a Key Player in the 'Coagulation-Atherogenesis' Maze? *Cardiovasc. Res.* **2009**, *82*, 392–403. [CrossRef]
63. Song, P.; Xie, J.; Li, W.; Zhang, X.; Sun, Z.; You, C. Effect of plasma thrombin-antithrombin complex on ischemic stroke: A systematic review and meta-analysis. *Syst. Rev.* **2023**, *12*, 17. [CrossRef]
64. Mo, J.H.; Liang, B.; Cen, J.T.; Li, W.Y.; Mo, Y.A.; Tang, M.C.; Ye, D.; Long, Q.X.; Hu, X.; Zhai, Y.S. Plasma thrombin-antithrombin complex as a candidate biomarker for coronary slow flow. *Front. Cardiovasc. Med.* **2025**, *12*, 1621655. [CrossRef]
65. Réganon, E.; Vila, V.; Martínez-Sales, V.; Vaya, A.; Aznar, J. Inflammation, Fibrinogen and Thrombin Generation in Patients with Previous Myocardial Infarction. *Haematologica* **2002**, *87*, 740–745.
66. Koyama, K.; Madoiwa, S.; Nunomiya, S.; Koinuma, T.; Wada, M.; Sakata, A.; Ohmori, T.; Mimuro, J.; Sakata, Y. Combination of Thrombin-Antithrombin Complex, Plasminogen Activator Inhibitor-1, and Protein C Activity for Early Identification of Severe Coagulopathy in Initial Phase of Sepsis: A Prospective Observational Study. *Crit. Care* **2014**, *18*, R13. [CrossRef]
67. Wu, C.H.; Yang, R.L.; Huang, S.Y.; Li, H.Z.; Wang, K.Y.; Yang, D.H.; Yan, X.H.; Xue, X.H.; Wu, S.Y.; Wang, J.M.; et al. Analysis of Thrombin-Antithrombin Complex Contents in Plasma and Hematoma Fluid of Hypertensive Intracerebral Hemorrhage Patients After Clot Removal. *Eur. J. Neurol.* **2011**, *18*, 1060–1066. [CrossRef]
68. Reda, S.; Schwarz, N.; Müller, J.; McRae, H.L.; Oldenburg, J.; Pötzsch, B.; Rühl, H. Fibrinolysis Biomarker, Thrombin, and Activated Protein C Level Alterations After Coagulation Activation Depend on Type of Thrombophilia and Clinical Phenotype. *Res. Pract. Thromb. Haemost.* **2024**, *8*, 102351. [CrossRef]
69. Di, W.; Xu, H.; Ling, C.; Xue, T. Early Identification of Lung Cancer Patients with Venous Thromboembolism: Development and Validation of a Risk Prediction Model. *Thromb. J.* **2023**, *21*, 95. [CrossRef]
70. Heerink, J.S.; Gemen, E.; Oudega, R.; Geersing, J.; Hopstaken, R.; Kusters, R. Performance of C-Reactive Protein, Procalcitonin, TAT Complex, and Factor VIII in Addition to D-Dimer in the Exclusion of Venous Thromboembolism in Primary Care Patients. *J. Appl. Lab. Med.* **2022**, *7*, 444–455. [CrossRef]
71. Meissner, M.H.; Zierler, B.K.; Bergelin, R.O.; Chandler, W.C.; Manzo, R.A.; Strandness, D.E., Jr. Markers of Plasma Coagulation and Fibrinolysis After Acute Deep Venous Thrombosis. *J. Vasc. Surg.* **2000**, *32*, 870–880. [CrossRef] [PubMed]
72. Committee for Medicinal Products for Human Use. *Guideline on Clinical Investigation of Recombinant and Human Plasma-Derived Factor IX Products*; European Medicines Agency: London, UK, 2011.
73. Baidildinova, G.; Nagy, M.; Jurk, K.; Wild, P.S.; Cate, H.T.; van der Meijden, P.E.J. Soluble Platelet Release Factors as Biomarkers for Cardiovascular Disease. *Front. Cardiovasc. Med.* **2021**, *8*, 684920. [CrossRef]
74. Banchereau, J.; Bazan, F.; Blanchard, D.; Brière, F.; Galizzi, J.P.; van Kooten, C.; Liu, Y.J.; Rousset, F.; Saeland, S. The CD40 Antigen and Its Ligand. *Annu. Rev. Immunol.* **1994**, *12*, 881–922. [CrossRef]
75. Zinellu, A.; Mangoni, A.A. sCD40 and sCD40L as candidate biomarkers of rheumatic diseases: A systematic review and meta-analysis with meta-regression. *Front. Immunol.* **2025**, *16*, 1479904. [CrossRef] [PubMed]
76. Antoniades, C.; Bakogiannis, C.; Tousoulis, D.; Antonopoulos, A.S.; Stefanadis, C. The CD40/CD40 Ligand System: Linking Inflammation with Atherothrombosis. *J. Am. Coll. Cardiol.* **2009**, *54*, 669–677. [CrossRef] [PubMed]
77. Prasad, K.S.S.; Andre, P.; Yan, Y.; Phillips, D.R. The Platelet CD40L/GP IIb–IIIa Axis in Atherothrombotic Disease. *Curr. Opin. Hematol.* **2003**, *10*, 356–361. [CrossRef]
78. Pamukcu, B.; Lip, G.Y.H.; Snezhitskiy, V.; Shantsila, E. The CD40–CD40L System in Cardiovascular Disease. *Ann. Med.* **2011**, *43*, 331–340. [CrossRef]
79. Nurden, A.T. Platelets, Inflammation and Tissue Regeneration. *Thromb. Haemost.* **2011**, *105*, S13–S33. [CrossRef] [PubMed]
80. Sanguigni, V.; Ferro, D.; Pignatelli, P.; del Ben, M.; Nadia, T.; Saliola, M.; Sorge, R.; Violi, F. CD40 Ligand Enhances Monocyte Tissue Factor Expression and Thrombin Generation via Oxidative Stress in Patients with Hypercholesterolemia. *J. Am. Coll. Cardiol.* **2005**, *45*, 35–42. [CrossRef]
81. Chen, C.; Chai, H.; Wang, X.; Jiang, J.; Jamaluddin, M.S.; Liao, D.; Zhang, Y.; Wang, H.; Bharadwaj, U.; Zhang, S.; et al. Soluble CD40 Ligand Induces Endothelial Dysfunction in Human and Porcine Coronary Artery Endothelial Cells. *Blood* **2008**, *112*, 3205–3216. [CrossRef]
82. Blumberg, N.; Gettings, K.F.; Turner, C.; Heal, J.M.; Phipps, R.P. An Association of Soluble CD40 Ligand (CD154) with Adverse Reactions to Platelet Transfusions. *Transfusion* **2006**, *46*, 1813–1821. [CrossRef]
83. Phipps, R.P.; Kaufmann, J.; Blumberg, N. Platelet-Derived CD154 (CD40 Ligand) and Febrile Responses to Transfusion. *Lancet* **2001**, *357*, 2023–2024. [CrossRef]
84. Chakrabarti, S.; Varghese, S.; Vitseva, O.; Tanriverdi, K.; Freedman, J.E. CD40 Ligand Influences Platelet Release of Reactive Oxygen Intermediates. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 2428–2434. [CrossRef]
85. Kappelmayer, J.; Nagy, B.; Miszti-Blasius, K.; Hevessy, Z.; Setiadi, H. The Emerging Value of P-Selectin as a Disease Marker. *Clin. Chem. Lab. Med.* **2004**, *42*, 475–486. [CrossRef]

86. Jaumdally, R.J.; Varma, C.; Blann, A.D.; MacFadyen, R.J.; Lip, G.Y.H. Indices of Angiogenesis, Platelet Activation, and Endothelial Damage/Dysfunction in Relation to Ethnicity and Coronary Artery Disease: Differences in Central Versus Peripheral Levels. *Ann. Med.* **2007**, *39*, 628–633. [CrossRef]
87. Chung, T.; Connor, D.; Joseph, J.; Emmett, L.; Mansberg, R.; Peters, M.; Ma, D.; Kritharides, L. Platelet Activation in Acute Pulmonary Embolism. *J. Thromb. Haemost.* **2007**, *5*, 918–924. [CrossRef] [PubMed]
88. Furio, E.; García-Fuster, M.J.; Redon, J.; Marques, P.; Ortega, R.; Sanz, M.J.; Piqueras, L. CX3CR1/CX3CL1 Axis Mediates Platelet-Leukocyte Adhesion to Arterial Endothelium in Younger Patients with a History of Idiopathic Deep Vein Thrombosis. *Thromb. Haemost.* **2018**, *118*, 562–571. [CrossRef]
89. Tsakiris, D.; Tschoepl, M.; Wolf, K.; Labs, K.; Jager, K.; Marbet, G. Platelets and Cytokines in Concert with Endothelial Activation in Patients with Peripheral Arterial Occlusive Disease. *Blood Coagul. Fibrinolysis* **2000**, *11*, 165–173. [CrossRef]
90. Riedl, J.; Hell, L.; Kaider, A.; Koder, S.; Marosi, C.; Zielinski, C.; Panzer, S.; Pabinger, I.; Ay, C. Association of platelet activation markers with cancer-associated venous thromboembolism. *Platelets* **2016**, *27*, 80–85. [CrossRef]
91. Hassani, S.; Fazeli, A.; Dorgalaleh, A.; Tabibian, S.; Lomei, J.; Alipour, M.; Kiani Zadeh, M.; Shams, M. Neutrophil-Mediated Effects on Hemostasis and Thrombosis: Unraveling Their Complex Interaction in Thrombotic Events. *Semin. Thromb. Hemost.* **2026**, *52*, 92–105. [CrossRef]
92. Fuchs, T.A.; Brill, A.; Duerschmied, D.; Schatzberg, D.; Monestier, M.; Myers, M.M., Jr.; Wrobleski, S.K.; Wakefield, T.W.; Hartwig, J.H.; Wagner, D.D. Extracellular DNA Traps Promote Thrombosis. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 15880–15885. [CrossRef] [PubMed]
93. von Brühl, M.L.; Stark, K.; Steinhart, A.; Chandraratne, S.; Konrad, I.; Lorenz, M.; Khandoga, A.; Tirniceriu, A.; Coletti, R.; Köllnberger, M.; et al. Monocytes, Neutrophils, and Platelets Cooperate to Initiate and Propagate Venous Thrombosis in Mice In Vivo. *J. Exp. Med.* **2012**, *209*, 819–835. [CrossRef]
94. Mangold, A.; Alias, S.; Scherz, T.; Hofbauer, M.; Jakowitsch, J.; Panzenböck, A.; Simon, D.; Laimer, D.; Bangert, C.; Kammerlander, A.; et al. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. *Circ. Res.* **2015**, *116*, 1182–1192. Erratum in *Circ. Res.* **2021**, *128*, e26. https://doi.org/10.1161/RES.0000000000000455. [CrossRef]
95. Medeiros, S.K.; Emery, B.; Bhagirath, V.; Parpia, S.; Dwivedi, D.J.; Dwivedi, N.J.; Kearon, C.; Liaw, P.C. Does Cell-Free DNA Promote Coagulation and Inhibit Fibrinolysis in Patients with Unprovoked Venous Thromboembolism? *Thromb. Res.* **2020**, *186*, 13–19. [CrossRef] [PubMed]
96. Smith, P.; Rosell, A.; Farm, M.; Bruzelius, M.; Gatica, G.A.; Mackman, K.; Odeberg, J.; Thålin, C. Markers of Neutrophil Activation and Neutrophil Extracellular Traps in Diagnosing Patients with Acute Venous Thromboembolism: A Feasibility Study Based on Two VTE Cohorts. *PLoS ONE* **2022**, *17*, e0270865. [CrossRef]
97. Jiménez-Alcázar, M.; Limacher, A.; Panda, R.; Méan, M.; Bitterling, J.; Peine, S.; Renné, T.; Beer, J.H.; Aujesky, D.; Lämmle, B.; et al. Circulating Extracellular DNA Is an Independent Predictor of Mortality in Elderly Patients with Venous Thromboembolism. *PLoS ONE* **2018**, *13*, e0191150. [CrossRef] [PubMed]
98. Martos, L.; Oto, J.; Fernández-Pardo, Á.; Plana, E.; Solmoirago, M.J.; Cana, F.; Hervás, D.; Bonanad, S.; Ferrando, F.; España, F.; et al. Increase of Neutrophil Activation Markers in Venous Thrombosis—Contribution of Circulating Activated Protein C. *Int. J. Mol. Sci.* **2020**, *21*, 5651. [CrossRef]
99. Borissoff, J.I.; Joosen, I.A.; Versteylen, M.O.; Brill, A.; Fuchs, T.A.; Savchenko, A.S.; Gallant, M.; Martinod, K.; Ten Cate, H.; Hofstra, L.; et al. Elevated Levels of Circulating DNA and Chromatin Are Independently Associated with Severe Coronary Atherosclerosis and a Prothrombotic State. *Arterioscler. Thromb. Vasc. Biol.* **2013**, *33*, 2032–2040. [CrossRef] [PubMed]
100. Langseth, M.S.; Opstad, T.B.; Bratseth, V.; Solheim, S.; Arnesen, H.; Pettersen, A.; Seljeflot, I.; Helseth, R. Markers of Neutrophil Extracellular Traps Are Associated with Adverse Clinical Outcome in Stable Coronary Artery Disease. *Eur. J. Prev. Cardiol.* **2018**, *25*, 762–769. [CrossRef]
101. Helseth, R.; Shetelig, C.; Andersen, G.; Langseth, M.S.; Limalanathan, S.; Opstad, T.B.; Arnesen, H.; Hoffmann, P.; Eritsland, J.; Seljeflot, I. Neutrophil Extracellular Trap Components Associate with Infarct Size, Ventricular Function, and Clinical Outcome in STEMI. *Mediat. Inflamm.* **2019**, *2019*, 7816491. [CrossRef]
102. Lim, H.H.; Jeong, I.H.; An, G.D.; Woo, K.S.; Kim, K.H.; Kim, J.M.; Yun, S.H.; Park, J.I.; Cha, J.K.; Kim, M.H.; et al. Evaluation of Neutrophil Extracellular Traps as the Circulating Marker for Patients with Acute Coronary Syndrome and Acute Ischemic Stroke. *J. Clin. Lab. Anal.* **2020**, *34*, e23190. [CrossRef]
103. Liu, J.; Yang, D.; Wang, X.; Zhu, Z.; Wang, T.; Ma, A.; Liu, P. Neutrophil Extracellular Traps and dsDNA Predict Outcomes Among Patients with ST-Elevation Myocardial Infarction. *Sci. Rep.* **2019**, *9*, 11599. [CrossRef]
104. Middleton, E.A.; He, X.Y.; Denorme, F.; Campbell, R.A.; Ng, D.; Salvatore, S.P.; Mostyka, M.; Baxter-Stoltzfus, A.; Borczuk, A.C.; Loda, M.; et al. Neutrophil Extracellular Traps Contribute to Immunothrombosis in COVID-19 Acute Respiratory Distress Syndrome. *Blood* **2020**, *136*, 1169–1179. [CrossRef] [PubMed]

105. Petito, E.; Falcinelli, E.; Paliani, U.; Cesari, E.; Vaudo, G.; Sebastiano, M.; Cerotto, V.; Guglielmini, G.; Gori, F.; Malvestiti, M.; et al. Association of Neutrophil Activation, More Than Platelet Activation, with Thrombotic Complications in Coronavirus Disease 2019. *J. Infect. Dis.* **2021**, *223*, 933–944. [CrossRef]
106. Arroyo, A.B.; Fernández-Pérez, M.P.; Del Monte, A.; Águila, S.; Méndez, R.; Hernández-Antolín, R.; García-Barber, N.; de Los Reyes-García, A.M.; González-Jiménez, P.; Arcas, M.I.; et al. miR-146a Is a Pivotal Regulator of Neutrophil Extracellular Trap Formation Promoting Thrombosis. *Haematologica* **2021**, *106*, 1636–1646. [CrossRef]
107. Wolach, O.; Sellar, R.S.; Martinod, K.; Cherpokova, D.; McConkey, M.; Chappell, R.J.; Silver, A.J.; Adams, D.; Castellano, C.A.; Schneider, R.K.; et al. Increased Neutrophil Extracellular Trap Formation Promotes Thrombosis in Myeloproliferative Neoplasms. *Sci. Transl. Med.* **2018**, *10*, eaan8292. [CrossRef]
108. Cruz, D.B.D.; Helms, J.; Aquino, L.R.; Stiel, L.; Cougourdan, L.; Broussard, C.; Chafey, P.; Riès-Kautt, M.; Meziani, F.; Toti, F.; et al. DNA-Bound Elastase of Neutrophil Extracellular Traps Degrades Plasminogen, Reduces Plasmin Formation, and Decreases Fibrinolysis: Proof of Concept in Septic Shock Plasma. *FASEB J.* **2019**, *33*, 14270–14280. [CrossRef]
109. Zifkos, K.; Dubois, C.; Schäfer, K. Extracellular Vesicles and Thrombosis: Update on the Clinical and Experimental Evidence. *Int. J. Mol. Sci.* **2021**, *22*, 9317. [CrossRef]
110. Abid Hussein, M.N.; Meesters, E.W.; Osmanovic, N.; Romijn, F.P.; Nieuwland, R.; Sturk, A. Antigenic Characterization of Endothelial Cell-Derived Microparticles and Their Detection ex vivo. *J. Thromb. Haemost.* **2003**, *1*, 2434–2443. [CrossRef] [PubMed]
111. Combes, V.; Simon, A.-C.; Grau, G.-E.; Arnoux, D.; Camoin, L.; Sabatier, F.; Mutin, M.; Sanmarco, M.; Sampol, J.; Dignat-George, F. In Vitro Generation of Endothelial Microparticles and Possible Prothrombotic Activity in Patients with Lupus Anticoagulant. *J. Clin. Investig.* **1999**, *104*, 93–102. [CrossRef]
112. Jimenez, J.J.; Jy, W.; Mauro, L.M.; Soderland, C.; Horstman, L.L.; Ahn, Y.S. Endothelial Cells Release Phenotypically and Quantitatively Distinct Microparticles in Activation and Apoptosis. *Thromb. Res.* **2003**, *109*, 175–180. [CrossRef] [PubMed]
113. Simoncini, S.; Njock, M.S.; Robert, S.; Camoin-Jau, L.; Sampol, J.; Harlé, J.R.; Nguyen, C.; Dignat-George, F.; Anfosso, F. TRAIL/Apo2L Mediates the Release of Procoagulant Endothelial Microparticles Induced by Thrombin In Vitro. *Circ. Res.* **2009**, *104*, 943–951. [CrossRef]
114. Bombeli, T.; Karsan, A.; Tait, J.F.; Harlan, J.M. Apoptotic Vascular Endothelial Cells Become Procoagulant. *Blood* **1997**, *89*, 2429–2442. [CrossRef] [PubMed]
115. Berckmans, R.J.; Nieuwland, R.; Böing, A.N.; Romijn, F.P.; Hack, C.E.; Sturk, A. Cell-Derived Microparticles Circulate in Healthy Humans and Support Low Grade Thrombin Generation. *Thromb. Haemost.* **2001**, *85*, 639–646. [CrossRef]
116. van Es, N.; Hisada, Y.; Di Nisio, M.; Cesarman, G.; Kleinjan, A.; Mahé, I.; Otten, H.M.; Kamphuisen, P.W.; Berckmans, R.J.; Büller, H.R.; et al. Extracellular Vesicles Exposing Tissue Factor for the Prediction of Venous Thromboembolism in Patients with Cancer: A Prospective Cohort Study. *Thromb. Res.* **2018**, *166*, 54–59. [CrossRef]
117. Jacintho, B.C.; Oliveira, J.D.; Césped, L.M.B.; de Souza, C.M.; Barion, B.G.; de Oliveira Vaz, C.; De Moraes Mazetto, B.; Orsi, F. Association Between Extracellular Vesicles (EVs) and Thrombosis in Antiphospholipid Syndrome. *Lupus* **2025**, *34*, 500–510. [CrossRef] [PubMed]
118. Antic, D.; Otasevic, V.; Mitrovic-Ajtic, O.; Djikic, D.; Zivkovic, E.; Dragojevic, T.; Ivanovic, J.; Stanisavljevic, D.; Milic, N.; Vukotic, M.; et al. Identifying Novel Coagulation Biomarkers for Thrombosis in Patients with Lymphoma. *Blood* **2023**, *142*, 4023. [CrossRef]
119. Schaller, J.; Gerber, S.S. The Plasmin-Antiplasmin System: Structural and Functional Aspects. *Cell. Mol. Life Sci.* **2010**, *68*, 785–801. [CrossRef]
120. Feinberg, W.M.; Macy, E.; Cornell, E.S.; Nightingale, S.D.; Pearce, L.A.; Tracy, R.P.; Bovill, E.G. Plasmin-Alpha2-Antiplasmin Complex in Patients with Atrial Fibrillation. *Thromb. Haemost.* **1999**, *82*, 100–103. [CrossRef]
121. Abdul, S.; Leebeek, F.W.; Rijken, D.C.; Uitte de Willige, S. Natural Heterogeneity of α2-Antiplasmin: Functional and Clinical Consequences. *Blood* **2016**, *127*, 538–545. [CrossRef]
122. Baráth, B.; Bogáti, R.; Miklós, T.; Kállai, J.; Mezei, Z.A.; Bereczky, Z.; Muszbek, L.; Katona, É. Effect of α2-Plasmin Inhibitor Heterogeneity on the Risk of Venous Thromboembolism. *Thromb. Res.* **2021**, *203*, 110–116. [CrossRef]
123. Ma, J.; Tang, Y.; Zhou, J.; Zhao, A.; Shi, J. Plasmin-Antiplasmin Complex as a New Predictive Marker of Postoperative Venous Thromboembolism in Patients with Gynecologic Malignancy. *Clin. Appl. Thromb./Hemost.* **2025**, *31*, 10760296251324918. [CrossRef]
124. Folsom, A.R.; Cushman, M.; Heckbert, S.R.; Rosamond, W.D.; Aleksic, N. Prospective Study of Fibrinolytic Markers and Venous Thromboembolism. *J. Clin. Epidemiol.* **2003**, *56*, 598–603. [CrossRef]
125. Tsikouras, P.; Deftereou, T.; Anthoulaki, X.; Bothou, A.; Chalkidou, A.; Christoforidou, A.; Chatzimichael, E. Thrombophilia and Pregnancy: Diagnosis and Management. In *Embolic Diseases—Evolving Diagnostic and Management Approaches*; IntechOpen: Rijeka, Croatia, 2020.
126. Patsouras, M.D.; Vlachoyiannopoulos, P.G. Evidence of Epigenetic Alterations in Thrombosis and Coagulation: A Systematic Review. *J. Autoimmun.* **2019**, *104*, 102347. [CrossRef]

127. Rask-Andersen, M.; Martinsson, D.; Ahsan, M.; Enroth, S.; Ek, W.E.; Gyllensten, U.; Johansson, Å. Epigenome-Wide Association Study Reveals Differential DNA Methylation in Individuals with a History of Myocardial Infarction. *Hum. Mol. Genet.* **2016**, *25*, 4739–4748. [CrossRef] [PubMed]
128. Li, J.; Zhu, X.; Yu, K.; Jiang, H.; Zhang, Y.; Deng, S.; Cheng, L.; Liu, X.; Zhong, J.; Zhang, X.; et al. Genome-Wide Analysis of DNA Methylation and Acute Coronary Syndrome. *Circ. Res.* **2017**, *120*, 1754–1767. [CrossRef] [PubMed]
129. Hollan, I.; Meroni, P.L.; Ahearn, J.M.; Tervaert, J.W.C.; Curran, S.; Goodyear, C.S.; Hestad, K.A.; Kahaleh, B.; Riggio, M.; Shields, K.; et al. Cardiovascular Disease in Autoimmune Rheumatic Diseases. *Autoimmun. Rev.* **2013**, *12*, 1004–1015. [CrossRef]
130. Whitaker, J.W.; Shoemaker, R.; Boyle, D.L.; Hillman, J.; Anderson, D.; Wang, W.; Firestein, G.S. An Imprinted Rheumatoid Arthritis Methylome Signature Reflects Pathogenic Phenotype. *Genome Med.* **2013**, *5*, 40. [CrossRef] [PubMed]
131. Chang, D.; Qian, C.; Li, H.; Feng, H. Comprehensive Analyses of DNA Methylation and Gene Expression Profiles of Kawasaki Disease. *J. Cell. Biochem.* **2019**, *120*, 13001–13011. [CrossRef]
132. Weeding, E.; Coit, P.; Yalavarthi, S.; Kaplan, M.J.; Knight, J.S.; Sawalha, A.H. Genome-wide DNA methylation analysis in primary antiphospholipid syndrome neutrophils. *Clin. Immunol.* **2018**, *196*, 110–116. [CrossRef]
133. Cuff, J.; Salari, K.; Clarke, N.; Esheba, G.E.; Forster, A.D.; Huang, S.; West, R.B.; Higgins, J.P.; Longacre, T.A.; Pollack, J.R. Integrative Bioinformatics Links HNF1B with Clear Cell Carcinoma and Tumor-Associated Thrombosis. *PLoS ONE* **2013**, *8*, e74562. [CrossRef]
134. Liu, X.; Tang, H.; Wang, Z.; Huang, C.; Zhang, Z.; She, X.; Wu, M.; Li, G. F10 Gene Hypomethylation, a Putative Biomarker for Glioma Prognosis. *J. Neurooncol.* **2012**, *107*, 479–485. [CrossRef]
135. Unruh, D.; Schwarze, S.R.; Khoury, L.; Thomas, C.; Wu, M.; Chen, L.; Chen, R.; Liu, Y.; Schwartz, M.A.; Amidei, C.; et al. Mutant IDH1 and Thrombosis in Gliomas. *Acta Neuropathol.* **2016**, *132*, 917–930. [CrossRef]
136. Augello, C.; Cattaneo, D.; Bucelli, C.; Terrasi, A.; Fermo, E.; Martinelli, I.; Iurlo, A.; Gianelli, U. CD18 Promoter Methylation Is Associated with a Higher Risk of Thrombotic Complications in Primary Myelofibrosis. *Ann. Hematol.* **2016**, *95*, 1965–1969. [CrossRef]
137. Hirahashi, J.; Hishikawa, K.; Kaname, S.; Tsuboi, N.; Wang, Y.; Simon, D.I.; Stavrakis, G.; Shimosawa, T.; Xiao, L.; Nagahama, Y.; et al. Mac-1 (CD11b/CD18) Links Inflammation and Thrombosis After Glomerular Injury. *Circulation* **2009**, *120*, 1255–1265. [CrossRef] [PubMed]
138. Sivapalaratnam, S.; Collins, J.; Gomez, K. Diagnosis of Inherited Bleeding Disorders in the Genomic Era. *Br. J. Haematol.* **2017**, *179*, 363–376. [CrossRef]
139. Lambert, M.P. Improving Interpretation of Genetic Testing for Hereditary Hemorrhagic, Thrombotic, and Platelet Disorders. *Hematol. (ASH Educ. Program)* **2020**, *2020*, 76–81. [CrossRef]
140. Ivaskevicius, V.; Windyga, J.; Baran, B.; Schroeder, V.; Junen, J.; Bykowska, K.; Seifried, E.; Kohler, H.P.; Oldenburg, J. Phenotype–Genotype Correlation in Eight Polish Patients with Inherited Factor XIII Deficiency: Identification of Three Novel Mutations. *Haemophilia* **2007**, *13*, 649–657. [CrossRef] [PubMed]
141. Biswas, A.; Ivaskevicius, V.; Thomas, A.; Oldenburg, J. Coagulation Factor XIII Deficiency. Diagnosis, Prevalence and Management of Inherited and Acquired Forms. *Hamostaseologie* **2014**, *34*, 160–166. [CrossRef]
142. Gresele, P.; Bury, L.; Falcinelli, E. Inherited Platelet Function Disorders: Algorithms for Phenotypic and Genetic Investigation. *Semin. Thromb. Haemost.* **2016**, *42*, 292–305. [CrossRef]
143. Gresele, P. Subcommittee on Platelet Physiology of the ISTH. Diagnosis of Inherited Platelet Function Disorders: Guidance from the SSC of the ISTH. *J. Thromb. Haemost.* **2015**, *13*, 314–322. [CrossRef]
144. Bastida Bermejo, J.M.; Hernández-Rivas, J.M.; González-Porras, J.R. Nuevos Métodos Diagnósticos en los Trastornos Plaquetarios Hereditarios. *Med. Clín.* **2017**, *148*, 71–77. [CrossRef]
145. Pezeshkpoor, B.; Oldenburg, J.; Pavlova, A. Experiences in Routine Genetic Analysis of Hereditary Hemorrhagic, Thrombotic, and Platelet Disorders. *Hamostaseologie* **2022**, *42*, S5–S12. [CrossRef]
146. Chaigneau, M.; Bowman, M.; Grabell, J.; Conboy, M.; Johnson, A.; Thorpe, K.; Guerin, A.; Dinchong, R.; Paterson, A.; Good, D.; et al. Genomic Testing for Bleeding Disorders (GT4BD): Protocol for a Randomised Controlled Trial Evaluating the Introduction of Whole Genome Sequencing Early in the Diagnostic Pathway for Patients with Inherited Bleeding Disorders Compared with Standard of Care. *BMJ Open* **2025**, *15*, e102041. [CrossRef] [PubMed]
147. Bonetti, C.; Silvestri, I.; Caccioppola, A.; Meli, A.; Grasselli, G.; Panigada, M. Viscoelastic Hemostatic Assays in Sepsis: From Pathophysiology to Potential Clinical Implications. *Semin Thromb Hemost.* **2025**. [CrossRef]
148. Swanson, E. Caprini Scores, Risk Stratification, and Rivaroxaban in Plastic Surgery: Time to Reconsider Our Strategy. *Plast. Reconstr. Surg.—Glob. Open* **2016**, *4*, e733. [CrossRef] [PubMed]
149. Zheng, Q.; Bai, J.; Fu, S.; Li, X.; Li, Y.; Zhou, Y.; Yu, J.; Gong, M.; Chen, D. Association Between Thrombelastography System and Vascular Obstructions and Hemorrhage: A Two-Year Follow-Up Study of Elderly Chinese Patients. *Ann. Clin. Lab. Sci.* **2014**, *44*, 194–201.

150. Tartamella, F.; Vassallo, M.C.; Berlot, G.; Grassi, P.; Testa, F. Thromboelastographic Predictors of Venous Thromboembolic Events in Critically Ill Patients: Are We Missing Something? *Blood Coagul. Fibrinolysis* **2016**, *27*, 804–811. [CrossRef]
151. Shreeve, N.E.; Barry, J.A.; Deutsch, L.R.; Gomez, K.; Kadir, R.A. Changes in Thromboelastography Parameters in Pregnancy, Labor, and the Immediate Postpartum Period. *Int. J. Gynecol. Obstet.* **2016**, *134*, 290–293. [CrossRef] [PubMed]
152. Sahli, S.D.; Castellucci, C.; Roche, T.R.; Rössler, J.; Spahn, D.R.; Kaserer, A. The Impact of Direct Oral Anticoagulants on Viscoelastic Testing—A Systematic Review. *Front. Cardiovasc. Med.* **2022**, *9*, 991675. [CrossRef]
153. De Pietri, L.; Bianchini, M.; Montalti, R.; De Maria, N.; Di Maira, T.; Begliomini, B.; Villa, E. Thrombelastography-Guided Blood Product Use Before Invasive Procedures in Cirrhosis with Severe Coagulopathy: A Randomized, Controlled Trial. *Hepatology* **2016**, *63*, 566–573. [CrossRef]
154. Roullet, S.; Freyburger, G.; Cruc, M.; Quinart, A.; Stecken, L.; Audy, M.; Sztark, F. Management of Bleeding and Transfusion During Liver Transplantation Before and After the Introduction of a ROTEM-Based Algorithm. *Liver Transplant.* **2015**, *21*, 169–179. [CrossRef]
155. Wikkelsø, A.; Wetterslev, J.; Møller, A.M.; Afshari, A. Thromboelastography (TEG) or Thromboelastometry (ROTEM) to Monitor Haemostatic Treatment Versus Usual Care in Adults or Children with Bleeding. *Cochrane Database Syst. Rev.* **2016**, *2016*, CD007871. [CrossRef]
156. Baksaas-Aasen, K.; Gall, L.S.; Stensballe, J.; Juffermans, N.P.; Curry, N.; Maegele, M.; Brooks, A.; Rourke, C.; Gillespie, S.; Murphy, J.; et al. Viscoelastic Haemostatic Assay Augmented Protocols for Major Trauma Haemorrhage (ITACTIC): A Randomized, Controlled Trial. *Intensive Care Med.* **2021**, *47*, 49–59. [CrossRef]
157. McNamara, H.; Kenyon, C.; Smith, R.; Mallaiah, S.; Barclay, P. Four Years' Experience of a ROTEM®-Guided Algorithm for Treatment of Coagulopathy in Obstetric Haemorrhage. *Anaesthesia* **2019**, *74*, 984–991. [CrossRef]
158. Deng, Q.; Hao, F.; Wang, Y.; Guo, C. Rotation Thromboelastometry (ROTEM) Enables Improved Outcomes in the Pediatric Trauma Population. *J. Int. Med. Res.* **2018**, *46*, 5195–5204. [CrossRef]
159. Knowles, L.M.; Wolter, C.; Menger, M.D.; Laschke, M.W.; Beyer, L.; Grün, U.; Eichler, H.; Pilch, J. Activation of the Acute-Phase Response in Hemophilia. *Thromb. Haemost.* **2023**, *123*, 867–879. [CrossRef]
160. Toenges, R.; Wittenbrink, A.; Miesbach, W. Biomarkers and Immunological Parameters in Haemophilia and Rheumatoid Arthritis Patients: A Comparative Multiplexing Laboratory Study. *Haemophilia* **2021**, *27*, e119–e126. [CrossRef]
161. Leptak, C.; Menetski, J.P.; Wagner, J.A.; Aubrecht, J.; Brady, L.; Brumfield, M.; Chin, W.W.; Hoffmann, S.; Kelloff, G.; Lavezzari, G.; et al. What Evidence Do We Need for Biomarker Qualification? *Sci. Transl. Med.* **2017**, *9*, eaal4599. [CrossRef]
162. Hatcher, H.; Stankeviciute, S.; Learn, C.; Qu, A.X. Regulatory, Translational, and Operational Considerations for the Incorporation of Biomarkers in Drug Development. *Ther. Innov. Regul. Sci.* **2025**, *59*, 519–526. [CrossRef] [PubMed]
163. Riva, N.; Vella, K.; Hickey, K.; Bertù, L.; Zammit, D.; Spiteri, S.; Kitchen, S.; Makris, M.; Ageno, W.; Gatt, A. Biomarkers for the Diagnosis of Venous Thromboembolism: D-Dimer, Thrombin Generation, Procoagulant Phospholipid and Soluble P-Selectin. *J. Clin. Pathol.* **2018**, *71*, 1015–1022. [CrossRef] [PubMed]
164. Zahra, Z.; Khalili, A.; Sharafi, M.; Mehrizi, R.; Ahadi, S.; Roshanshad, A.; Malakootian, M. Metabolomics Profiling in Venous Thromboembolism and Its Chronic Sequelae: A Systematic Review. *Thromb. Res.* **2025**, *249*, 109309. [CrossRef] [PubMed]
165. Tsimillis, K.C.; Michael, S. Accreditation of Medical Laboratories: What Is New in ISO 15189:2022. *Accredit. Qual. Assur.* **2024**, *29*, 175–182. [CrossRef]
166. Cui, W.; Xu, W.; Fan, Y.; Wang, X.; Zhu, J.; Qu, L. Application of Point-of-Care Testing for Coagulation Tests: Comparison of Consistency and Interference with a Fully Automatic Coagulation Analysis System. *iLABMED* **2025**, *3*, 349–358. [CrossRef]
167. Moser, K.A.; Smock, K.J. Direct Oral Anticoagulant (DOAC) Interference in Hemostasis Assays. *Hematol. (ASH Educ. Program)* **2021**, *2021*, 129–133. [CrossRef]
168. Gosselin, R.C.; Adcock, D.M. Assessing Direct Oral Anticoagulants in the Clinical Laboratory. *Clin. Lab. Med.* **2024**, *44*, 551–562. [CrossRef]
169. Vasan, R.S. Biomarkers of cardiovascular disease: Molecular basis and practical considerations. *Circulation* **2006**, *113*, 2335–2362. [CrossRef]
170. Martins, F. Innovative Biomarkers for Early Detection of Rare Hematological Disorders. *J. Rare Disord. Diagn. Ther.* **2024**, *10*, 191.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

https://doi.org/10.3390/biom16020235