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Factors Contributing to the Risk of Venous Thromboembolism

Yihang Chen[1] and Ali Alamdar Shah Syed[#]

[1] Shanghai American School Puxi, China
[#]Advisor

ABSTRACT

Both the deep vein thrombosis and pulmonary embolism are classified into venous thromboembolism (VTE). Risk factors for VTE are multifactorial with both genetic and non-genetic risk factors. Factor V Leiden, Prothrombin, and ABO blood group are three widely researched mutations highly associated to VTE while there are other rarer genetic risk factors such as Protein C, Protein S, Antithrombin deficiencies. Race and ethnicity, age, sex, diet and lifestyle are examples of some of the non-genetic risk factors for VTE; however, because non-genetic risk factors may be dependent of other environmental factors (such as the relationship between sex and oral contraceptives), their individual association to VTE needs further research.

Introduction

Venous thromboembolism (VTE) is characterized by blood clots developing in the veins, leading to disruption of blood flow. VTE can be categorized into two main types, deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT usually occurs in the limbs, most commonly in the legs, while in PE the blood clot detaches from its initial position and makes its way to the lungs where it disrupts blood flow in the pulmonary artery. The global incidence of VTE is approximately 10 million cases per year, of which about 60% can be attributed to DVT, while PE makes up the remaining 40% of cases. VTE is a major burden on healthcare systems globally, both in terms of incidence and cost of treatment [1]. A report from the US surgeon general predicts the mean cost for hospitalization of VTE patients at $62,838, which is approximately 2.5 times higher than the mean cost of active cancer hospitalizations ($24,464) [2]. Hospitalization rates of VTE have steadily increased over the past two decades, further exuberating the economic burden placed on society [3]. Overall mortality of VTE has seen a decline due to advances in the quality and availability of healthcare, but 9.2% deaths in pregnant women are caused by PE, making VTE a serious contributor to maternal morbidity and mortality [4]. Furthermore, VTE is the leading preventable cause of death in hospitalized patients [5] and it is therefore essential to understand the factors that contribute to VTE risk.

VTE is a multifactorial disorder that is approximately 50-60% of the heritability attributed to genetic factors [6] and is therefore considered a complex interaction of genetic and environmental factors. This review aims to shed light on the risk factors of VTE which may be genetic, mainly Factor V Leiden, prothrombin, ABO blood group, protein C deficiency, protein S deficiency, and antithrombin deficiency – or non-genetic in nature. The review is limited to VTE and does not include Arterial thromboembolism (ATE).

Genetic risk factors for VTE

The genetics revolution of the late 20th and early 21st centuries has brought to light new insights into human diseases, including VTE for which the first genetic associations were made by Olav Egeberg while studying a

Scandinavian family suffering from DVT[6], propelling thrombophilia into the limelight and establish the study of thrombosis as an import clinical discipline [7]. It wasn't until 1981 that other associations were made, when protein S and C deficiencies were detected in families suffering from VTE [8].

Factor V Leiden

The arrival of sequencing technology enabled the detection of a mutation in the Factor V gene that caused poor anticoagulant response [9]. Factor V Leiden (FVL) is a mutation at nucleotide 1691 in the *FV* gene: a guanine to adenine substitution (position 506) also known as the SNP rs6025. Found in around 4-5% of the population, this mutation is known to be the strongest genetic variant related to VTE. FVL causes resistance to the protein C (APC) pathway, an anticoagulant pathway that serves to control thrombosis and inflammatory responses [10]. In this pathway a transmembrane glycoprotein, thrombomodulin binds to thrombin to inactivate the coagulation process and at the same time activate protein C. Endothelial cell protein C receptor (EPCR) also binds to protein C to amplify protein C activation. Once the activated protein C detaches, it binds to protein S where it will inactivate factor Va, a product of the cleavage of factor V, decreasing thrombin synthesis.

In summary, the role of protein C is to cleave the coagulation factor V (coded by the *FV* gene) to inactivate the coagulation process and slow down the clotting process. However, with FVL, factor V becomes insensitive to the natural anticoagulant protein C and therefore thrombin levels will increase, increasing the risk for VTE [8]. Carries of a single copy of the FVL mutation have a 10-fold increased risk of developing VTE, while risk rises to up to 140-fold for homozygous individuals [8, 9].

Interestingly FVL VTE has an 11.8–20% increased incidence of upper limbs DVT [9], with individuals showing a surprisingly lower relative risk of PE (only 1.7 compared to 4.5 for DVT [11]. Recent studies have suggested that this 'FVL paradox' is only associated with heterozygotes for FVL [12, 13]. However, the underlying mechanism of this paradox is yet to be understood.

Prothrombin

Prothrombin is a protein synthesized in the liver, which is cleaved by the membrane dependent prothrombinase complex, to form thrombin [14]. Thrombin is an essential coagulation factor which activates factor V, factor VIII, factor XI (other coagulation factors) and cleaves fibrinogen to form the protein firbin [8]. Firbin gets polymerized to entangle platelets in the plasma to form a clot at wound sites. The prothrombin G20210A mutation is a guanine to adenine substitution at the position 20210 (SNP rs1799963) of the 3' untranslated region of the prothrombin gene (*F2*), resulting in an increase in gene expression and a 133% increase in serum prothrombin levels. This mutation also increases the risk of VTE by 2-4 fold [8, 15]. Specifically, there is a 2-3 fold and a 5-10 fold increased risk of VTE in heterozygotes and homozygotes respectively [8]. A guanine to thymine substitution resulting in a arginine to leucine substitution at position 596 in the prothrombin gene was first detected in a 17 year old Japanese girl with DVT and was named prothrombin Yukuhashi [16]. This mutation only causes a slightly impaired procoagulant function of the mutant prothrombin than the wild type, it still shows significantly increased thrombotic risk [16]. Takagi et al dived into the pathway of prothrombin Yukuhashi that causes VTE; it demonstrated that prothrombin Yukuhashi may promote thrombomodulin resistance which increases the risk for VTE [17].

Bank et al investigated the link between the incidence of prothrombin mutation and pregnancy complications, with rs1799963 found in around 17% of pregnant women with VTE, there is no evidence of the mutation being associated with pregnancy complications [18, 19]. One study by the Eunice Kennedy Shriver

National Institute of Child Health and Human Development sampled more than 4,167 women in the first trimester for the prothrombin mutation but only a total of 3.8% of women carried the mutation; the results of the study showed no association between pregnancy and the SNP rs1799963 [18]. More research is required to understand the role or lack thereof of prothrombin mutations in pregnancy complications attributed to VTE.

ABO Blood Group

The ABO blood group is a major risk factor to VTE, because of ABO's relation to the procoagulant factor VIII and the Van Willebrand factor (VWF), which has two biological forms: high molecular weight (HMW) and low molecular weight (LMW) [20]. HMW VWF mediates platelet adhesion and aggregation while the LMW VWF localizes factor VIII (a blood-clotting protein coded in the F8 gene) to a site of injury. The relationship with the ABO blood group and VWF is that A, B, and H antigens are present on the surface of VWF and are close to the A2 domain binding site for ADAMTS13 — a protease that cleaves HMW VWF to LMW VWF. These antigens may affect the affinity between ADAMTS13 and HMW VWF and decrease the rate of cleavage [20]. Decreasing cleavage will lead to an accumulation of HMW VWF or also called ultra-large VWF which will lead to spontaneous interactions with platelets and cause thrombosis [21]. This may be why type O blood groups have around 25% lower VWF levels, but VWF factors are also influenced by factors other than ABO blood groups [22]. Further research is needed to fully grasp ABO blood group's impact on VWF levels. Nevertheless, the literature shows that non-O ABO blood types have an 86% (95% CI: 1.35, 2.57) increased risk for VTE [23]. Furthermore, even though ABO blood groups and VWF are important factors that regulate factor VIII plasma levels, factor VIII is also effected by environmental factors such as liver cirrhosis [20]. Factor VIII has been generally accepted to be an independent VTE risk factor without relation to VWF; while a study by Morange et al has shown that ABO group still has an effect on Factor VIII after accounting for adjusted VWF levels [24].

A meta-analysis by Dentali et al. (comprised of 38 studies with 10,305 VTE cases) concluded that the non-O blood type is the single greatest genetic risk factor for VTE with an OR of 2.09 ($p < 0.00001$) [25]. However, this study could not test for the prothrombin mutation because of a lack in the number of studies on the mutation.

Protein C, Protein S, and Antithrombin Deficiency

Protein C (PC) is a vitamin K dependent glycoprotein that is encoded in the *PROC* gene in chromosome 2 and is produced in the liver. It is inactive circulating in the blood plasma until it binds to thrombomodulin, and the activated PC will inactivate factor Va and VIIIa inducing thrombin synthesis [26]. Therefore, a deficiency in PC will increase the risk of VTE. There are many different mutations (almost 200) in the *PROC* gene that can lead to PC deficiency: for example, the Gly197 to Arg mutation in the *PROC* gene [8, 27]. However, only 0.2% of the population carries the risk allele, and the deficiency only accounts for 2–5 % of VTE patients [28, 29].

Protein S (PS) is another vitamin K dependent glycoprotein that also circulates in the plasma in either a form bound to a protein, attached to the C4b-binding protein, a complement regulator protein or free form. Only PS's free form serves as the cofactor for PC, increasing activation resulting in increased cleavage of factor Va by around10 fold [30]. Therefore, mutations in the *PROS* gene will decrease PC's ability to inactivate factor Va and increase thrombin synthesis and chances of VTE. There are three different types of PS deficiency: type I, type II, and type III. Type I has a decreased level of bound and free forms of PS; Type II has normal levels of bound and free forms of PS, but the function is altered; Type III has normal levels of bound PS but decreased level of free PS [8]. All three types are rare in the population and difficult to diagnose, but research has shown PS deficiency in around 1–13% of VTE patients [31]. Similar to PC, there have been almost 200 mutations discovered in the *PROS* gene such as the PS Heerlen and the PS Tokushima mutations, resulting in to type II

and III PS deficiencies respectively [30]. The rarity coupled with multiple types of PS has made research into the relationship between VTE and PS difficult, however low levels of free PS have been shown to increase the risk for VTE [8].

A vitamin K independent glycoprotein, Antithrombin (AT) is a major inactivator of factor Xa, which prevents the generation of thrombin and is plays an important role in the coagulation system. AT is encoded in the *SERPINC1* gene and there are more than 130 known mutations that cause two different types of AT deficiency: type I (reduced levels of AT) and type II (dysfunctional AT). Deletions in the codons 81, 106, 107, 244, and 245 of *SERPINC1* are associated with type I AT deficiency. Single base pair substitutions are the likely cause for type II AT deficiency such as in the regions Ala382 and Ala384 [32]. A deficiency of AT results in an increased risk of VTE, but the chances of inheriting AT deficiency is extremely low (1 in 500-5000) and is mostly a result of other conditions such as liver diseases, chemotherapy, and nephrotic syndrome [8, 33, 34].

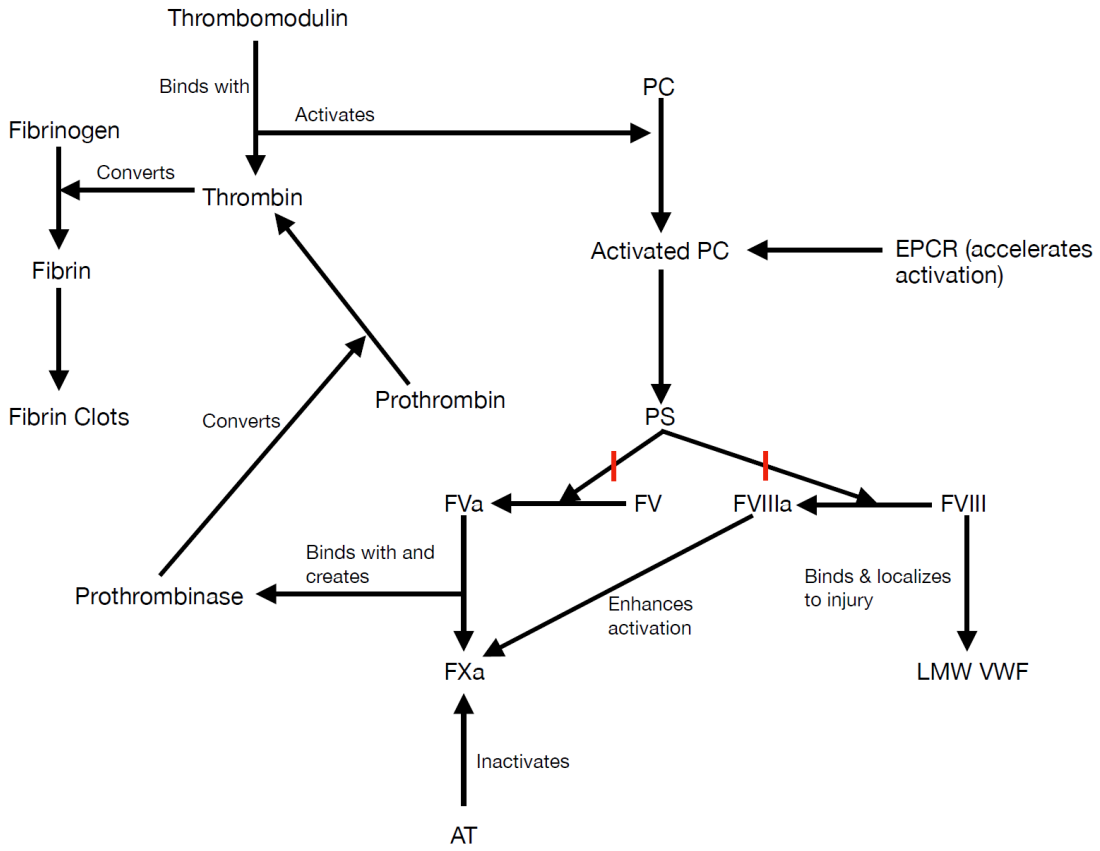

Figure 1. Interactions between all the factors and proteins involved in the coagulation system eventually leading to fibrin clots and an increase in susceptibility to VTE

Other Genes Associated with VTE

Over the years genes other than the ones described above have been associated with VTE such as the Methylenetetrahydrofolate reductase (*MTHFR*) gene which codes for the enzyme MTHFR. Mutations such as *MTHFR* C677T decreases the MTHFR enzyme activity which leads to hyperhomocysteinemia [35]. An increased level of homocysteine leads to increased thrombin generation and greater factor V activity which increases the chances for VTE [36]. However, the MTHFR – Hyperhomocysteinemia – VTE relationship is still ambiguous

as a study found that polymorphisms in the *MTHFR* gene do not correlate with hyperhomocysteinemia, and neither does hyperhomocysteinemia correlate with VTE risk, but *MTHFR* genotypes provide a robust assessment of VTE risk [37]. Further research is required to better understand how MTHFR contributes to VTE risk independent of hyperhomocysteinemia. Figure 1 summarizes the interactions between factors and proteins in the coagulation system which in turn increases or decreases the risk for VTE. The read marker describes an inhibition while the arrows describes and inactivation, activation, binding, or conversion.

Insights from Genome Wide Association Studies

Genome wide association studies (GWAS) over the past few decades have been employed to better understand the genetic variants that contribute to disease risk, with multiple meta-analyses revealing a successively greater number of variants associated with VTE [38-40]. The latest meta-analysis consists of 30234 cases and 172122 controls; the results of which show that the variants that are most significantly associated with VTE lay in *F5*, *ABO*, *F11* and *FGG* regions, all classically associated with VTE. GWAS studies have discovered novel risk genes for VTE as well, such as *SCARA5*, *STX10*, and *SMG6* to name a few, however the associated SNPs are present in non-coding intronic regions. Table 1 displays a full list of the SNPs associated with VTE identified by GWAS meta-analyses.

Table 1. Top 10 SNPs associated with VTE [41]

Chromosome:Position	**Variant identifier: effective/reference allele (amino acid substitution)**	**Trans-Ancestry Odd Ratio**	**Trans-Ancestry *P* value**	**Locus (location)**
1:169519049	rs6025: T/C (Arg>Gln)	2.39 (2.25-2.53)	1.4E-188	*F5* (exon)
9:136137106	rs687289: A/G	1.34 (1.31-1.36)	1.3E-174	*ABO* (intron)
9:136141870	rs2519093: T/C	1.40 (1.37-1.43)	3.8E-169	*ABO* (intron)
9:136154168	rs579459: C/T	1.36 (1.33-1.39)	3.9E-145	*ABO* (intron)
4:187207381	rs2289252: T/C	1.19 (1.16-1.21)	3E-65	*F11* (intron)
4:155525695	rs2066864: A/G	1.20 (1.17-1.22)	2E-59	*FGG* (intron)
4:187192481	rs2036914: T/C	0.86 (0.84-0.87)	1.6E-54	*F11* (intron)

4:187204937	rs4253421: A/G	0.80 (0.78-0.83)	8.1E-41	*F11* (intron)
11:46933311	rs191945075: A/G	1.86 (1.68-2.07)	9.5E-32	*LRP4* (intron), *F2* (downstream)
9:136131188	rs8176749: T/C (Leu>Leu)	1.23 (1.19-1.28)	2.3E-30	*ABO* (exon)

Non-genetic Risk Factors for VTE

Race and Ethnicity

A study looking into VTE diagnosis incidence in 1996 showed that African Americans had the highest incidence of diagnosis, with 141/100,000 adults diagnosed per year, followed by Caucasians with 103/100,000, Hispanics with 61.5/100,000 and Asians with only 29/100,000 adults diagnosed per year respectively [42]. Furthermore, African Americans show a 56% higher incidence of secondary VTE than Caucasians. Results from the Cardiovascular Health Study, a large cohort study begun in 1988 show African Americans have an 81% increased risk of developing VTE than caucasians (CI: 1.20, 2.83) [43] and the Medicare database in the US shows about 5.8 cases per 1000 African Americans with incidence of VTE compared to 4.6 cases per 1000 Caucasians [44]. However, paradoxically the FVL and prothrombin mutations that are the main risk factors for VTE, are more prevalent in the Caucasian than the African American population [45]. Researchers from the Atherosclerosis Risk in Communities Study couldn't find a significant difference in VTE susceptibility between African Americans and Caucasians (CI: 0.96, 1.54) [43]. Further research may be required to address these inconsistencies, which may be due the Africans possessing unique risk variants and the over representation of the Caucasian population in such scientific studies. Indeed, data has emerged that shows VTE incidences in Asia are similar to those in the United States [46].

Age

The risk of VTE increases with age, young adults show VTE incidences of 0.5-1 event per 1000 person per year [47]. However, individuals aged 80 and above experience about 5-7 VTE related events per 1000 person per year [47, 48]. It has been proposed that the levels of clotting factors such as IX, XI and VIII increase with age, which would result in an increase in blood coagulability thereby explaining the increased incidence of VTE. Factor IX and XI antigen levels above the 90th percentile increased the risk for DVT by 2.8-fold and 2.2-fold respectively [49]. Furthermore, research by Biguzzi et al used linear regression of 2923 individuals (after excluding cancerous individuals and pregnant women) to analyze the relationships between age and VWF levels, showing that there is an increase in VWF and factor VIII activity with age [50]. VWF circulates in the plasma with factor VIII promoting platelet adhesion and thrombus formation; therefore, an increase in factor VIII increases the risk for VTE. Interestingly, carriers of non-O blood group showed a higher increase of factor VIII activity levels with an increase in age[50].

Other important considerations are aging related comorbidities such as diabetes mellitus, chronic obstructive pulmonary disease and hypertension that are risk factors for VTE [51]. Research shows that cancer is an independent risk factor to VTE with a prevalence of 7.8% in cancer patients, however the risk of a cancerous

diagnosis decreased in patients above 65 years old during the first year of follow up after a VTE incident[52, 53]. As age increases it is important to pay more attention to VTE related biomarkers and assays.

Sex

Women between 20 and 44 years of age show a higher risk of VTE than men [54]. However, this increase in risk may be due to the use of oral hormonal contraceptives and pregnancy: 1 in 2-4 women incidences of VTE in women may be attributed to oral contraceptives [55]. During pregnancy levels of fibrinogen, factor VIII and VWF all increase, thereby increasing risk of VTE [56].

After adjusting for the above reproductive risk factors, studies show that men have double the risk of VTE compared to women (OR = 2.1; 95% CI: 1.9, 2.4) [57, 58]. Another study shows age adjusted risk for VTE is 130 and 110 per 100,000 person per years in men and women respectively [47]. The difference in the risk for VTE in men and women may be mediated by body height, as a recent study has found that those who were taller than 178cm has a two-fold higher risk for VTE than those who were shorter than 165cm (95% CI = 1.51-2.73) [59]. However, a recent systematic review on the trends of DVT in the general population failed to find a significant difference in the risk for DVT in men and women [60], while another study investigating middle aged populations found an increase in risk for DVT in men than women (95% CI: 0.75, 1.50) [61]. Taken together, the literature seems to have formed a consensus that men are more susceptible to VTE than women, however pregnancy and oral contraceptives play an important role in the development of VTE in women and should increase screening and monitoring for VTE regarding these risks. Further research is required to identify the causes of an increase in VTE risk in men, as well as sex-related association to specific types of VTE such as DVT and PE.

Diet and Lifestyle

There have been several studies into the effect of diet on VTE risk, however most of these studies have identified insignificant or conflicting relationships. A study published in 2009 showed that coffee, diet soda and fish consumption were associated with an increased VTE risk; however, the relationships became insignificant when accounting for BMI and diabetes [62]. The study went on to report that there was no significant relationship between western dietary patterns, vitamin E, vitamin B6 and VTE, contrary to results from another study by Steffan et al which reported that western dietary patterns were associated with higher risk of VTE when compared to plant and fish-based diets [63]. A more recent study published in 2021 found that western dietary patterns increased the risk of VTE in men but not women and reported positive relationships between vitamin E and vitamin B6 and the risk of VTE [64]. Smoking has been associated with an increase in VTE risk, but anti-inflammatory food consumption has been shown to decrease the risk of VTE in smokers [65, 66]. Daily alcohol consumption reduced the risk of VTE [62]. Another study from Gregson et al collected data from the Emerging Risk Factors Collaboration (ERFC) with 731,728 participants concluded that smoking and adiposity (adiposity was more associated to PE than DVT) were both risk factors that increased susceptibility to VTE, while there was inconsistent associations between diabetes and blood pressure with VTE risk [67]. Furthermore, there is evidence to show that surgery, obesity, cystic fibrosis, sepsis, systemic infection, cancer, inflammatory bowel disease, and lupus may increase susceptibility to VTE through inflammatory mediators that modulate thrombosis [68].

Physical activity and its relationship to VTE risk is uncertain and holds mixed results. According to a study by Stralen et al, physical activity modeled as a time-varying exposure increases the risk for VTE (HR(adj)=1.38, 95% CI=0.99-1.91) when adjusted for sex, age, race, self-reported health, and body mass index[68]. This study further shows that non-strenuous physical activity has no association to VTE (HR(adj)=0.75,

95% CI=0.49-1.16) while strenuous activity increased VTE risk (HR(adj)=1.75, 95% CI=1.08-2.83) in comparison to no physical activity. On the other hand, a large population-based case control study (MEGA study) by the same writer concluded that an active participation in sports reduced the risk for VTE (OR=0.71; 95% CI: 0.64, 0.78) [69]. In support, a more recent study from Johansson et al of 108,025 participants concludes that there is a lower risk of first time VTE in women with increased physical activity. Furthermore, Borch et al analyzed 26,490 middle-old aged (25-97 years old) participants and found no relationship between moderate intense physical activity and the risk of VTE [70]. Evidently, there are conflicting conclusions to the risk of physical activity and VTE risk; therefore, further studies are required to assess the effect of physical activities to VTE risk as well as how age/sex plays a role in the relationship between physical activity and VTE.

Conclusion

In conclusion, VTE risk is a multifactorial combination of both genetic and non-genetic risk factors. The genetic risk factors of VTE such as FVL and *F2* genes are well studied, but more research needs to be conducted in order to uncover the causes of FVL and prothrombin mutation independent VTE in African populations and better understand the gender related differences in VTE incidence. New variants are still being researched from the well-researched genes as well as new loci in meta-analyses of exome-wide VTE association studies. Furthermore, VTE is a preventable disease that can be diagnosed. Primarily, patients would receive a clinical pre-test probability using scoring systems such as the Wells score or Geneva score (for PE) [71]. This pre-test probability would most likely be high with some of the risk factors mentioned in the non-genetic risk factors for VTE. There would be more tests after the pre-test probability such as using D-dimer assays: whole-blood agglutination assays, immunosorbent assays, and latex agglutination assays [2]. D-dimer is a fibrin degradation product that can be measured in the plasma. Those with thrombosis would carry a high level of circulating D-dimer, and this biomarker helps to identify those with a high risk of VTE. Taken together, clinicians should identify and communicate with individuals that are at risk of developing VTE to raise awareness and take measures in order to reduce this preventable cause of death.

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