Defective Fibrinolysis and Genetic or Acquired Pro-coagulant Changes Synergistically Increase the Risk of Venous Thrombosis

a report by

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Venous thrombosis has a high incidence of around one per 1,000.[1] The first phase of the disease, a deep vein thrombosis (DVT) of the leg or pulmonary embolism, requires pharmacological treatment and may require hospitalisation. About 20–50% of patients develop 'post-thrombotic syndrome' within one to two years of their first venous thrombosis. This causes pain, ulceration and limb swelling and requires medical attention.[2,3] Venous thrombosis may be life-threatening. Over 10% of patients with venous thrombosis (without cancer) die within one year.[1] Despite treatment of a primary DVT or pulmonary embolism, patients remain at high risk of recurrence. Cumulative recurrence rates of 3–13% after one year and 12–28% after five years following treatment cessation have been reported.[1,2,4]

Risk Factors for Venous Thrombosis and a Model of Thrombotic Risk

In 1856, Virchow postulated his triad proposing three main distinct mechanisms that may result in thrombosis: alterations in normal blood flow or stasis; alterations in the constitution of blood or hyper-coagulability; and vascular damage.[5] The first two mechanisms are thought to be most relevant in the development of a venous thrombosis.

There are many well-known risk factors for venous thrombosis and these have been extensively studied. Risk factors can either be acquired, such as oral contraceptive use or surgery, or have a genetic origin. Genetic risk factors such as factor V Leiden, prothrombin 20210A or deficiencies of the natural anticoagulants antithrombin, protein-S or protein-C are all known to be associated with an increased capacity to form a blood clot.[6] It has been demonstrated that the occurrence of multiple risk factors in a single individual can result in a risk of thrombosis exceeding the sum of the individual risks. In other words, two factors can have synergistic effects on the risk of venous thrombosis. A well-known example is the combination of oral contraceptive use and factor V Leiden. Women who take oral contraceptives and do not carry the factor V Leiden mutation have an approximately four-fold increased risk of venous thrombosis. Factor V Leiden increases the risk to approximately five-fold in a woman not taking oral contraceptives. Carriers of factor V Leiden who also use oral contraceptives have an estimated 30-fold increased risk of venous thrombosis.[7]

A model to describe thrombotic risk potential has been previously proposed. This model incorporated age and acquired and/or genetic risk factors and the interaction or synergy between them.[6] It assumes each risk factor to cumulatively contribute to the potential to precipitate a venous thrombotic event. There is a certain risk threshold; when this threshold is reached, a person will suffer a thrombotic event (see *Figure 1*).

Figure 1A shows the thrombosis potential of a woman during her lifetime. The black lines indicate the thrombosis potential for each individual risk factor when no other risk factors are present. This woman is a carrier of the factor V Leiden mutation, had an intravenous catheter in her youth, used oral contraceptives during a certain period and became immobilised as a result of a skiing accident. The result of these risks, when all these individual risks are added up, is shown in purple. In *Figure 1B*, this purple line is shown together with the line that represents the thrombosis threshold. In this example, the woman will develop venous thrombosis at 30 years of age when she takes oral contraceptives and becomes immobilised. The interaction between factor V Leiden and oral contraceptives is not taken into account. This effect is shown in an additive way. In *Figure 2B* the purple line shows the risk potential when this synergy is taken into account (see *Figure 2A*).
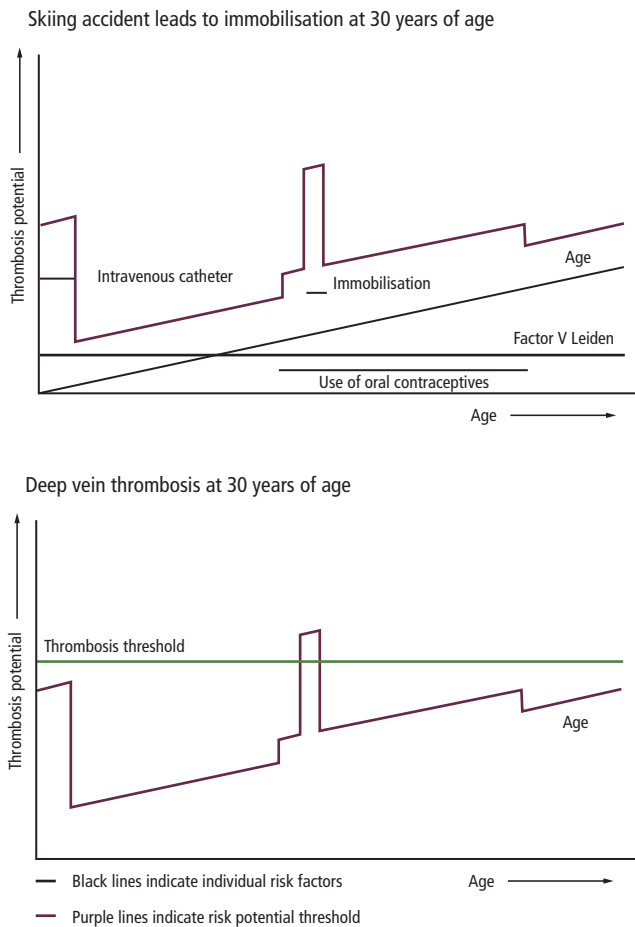
Hypofibrinolysis as a Risk Factor for Venous Thrombosis

Several genetic and acquired risk factors for venous thrombosis are associated with a hypercoagulable state (i.e. an increased capacity to form a blood clot). Little information is available on the association between hypofibrinolysis (i.e. a decreased capacity to dissolve a clot) and the risk of venous thrombosis. Fibrinolytic activity is defined as the capacity of plasmin, the activated form of plasminogen, to dissolve a fibrin clot into fibrin degradation products (see *Figure 3*).

The main activators of plasminogen are urokinase and tissue-type plasminogen activators (u-PA and t-PA). The main activator in plasma is t-PA, which is produced in vascular endothelial cells and locally released into the circulation upon stimulation.[8] Plasminogen activator inhibitor (PAI-1) is the most important inhibitor of t-PA and functions by forming a firm 1:1 complex with t-PA, which is then cleared by the liver.[9,10] The main physiological plasmin inhibitor in plasma, α_2-antiplasmin, is a serine protease that directly inhibits plasmin by the formation of a stable inactive complex.[11] Thrombin-activatable fibrinolysis inhibitor (TAFI) can be seen as the molecular connection between the coagulation and fibrinolytic cascades. TAFI is activated most effectively by the thrombin/thrombomodulin complex and attenuates fibrinolysis by removing carboxy-terminal lysine and arginine residues from partially degraded fibrin. This decreases the ability of plasminogen and t-PA to bind to the fibrin surface, resulting in a decrease in plasmin formation and a reduction in clot degradation.[12]

Although it is biologically plausible that decreased fibrinolytic potential increases the risk of venous thrombosis, this mechanism has not been studied as extensively as hypercoagulability. One important observation argues against a role for the fibrinolytic system in venous thrombosis: individuals who are plasminogen-deficient do not appear to have an increased risk of venous thrombosis.[13] Plasminogen-deficient patients

Figure 1: Model of Thrombosis Risk[6]

Skiing accident leads to immobilisation at 30 years of age

Deep vein thrombosis at 30 years of age

— Black lines indicate individual risk factors
— Purple lines indicate risk potential threshold

Figure 2: Models of Thrombosis Risk with Different Interactions Between Factor V Leiden and the Use of Oral Contraceptives[6]

Additive effects

Supra-additive effect

suffer from ligneous conjunctivitis, which is rare in the general population. It is characterised by the development of firm fibrin-rich, pseudo-membranous lesions primarily on the tarsal conjunctivae.[13]

The relationship between plasma α_2-antiplasmin levels and venous thrombosis has not been extensively studied. Plasma levels of t-PA and PAI-1 levels do not seem to influence thrombotic risk.[14–16] Studies on PAI-1 levels have yielded conflicting results.[17,18] In the 1980s various epidemiological studies were undertaken using assays to determine overall fibrinolytic potential, such as the euglobulin clot lysis time (ECLT) and dilute whole blood clot lysis time (DWBCLT). As the CLT measured by these assays did not seem to be related to the risk of venous thrombosis,[19] the interest in fibrinolysis decreased and the dissolution of the blood clot was put to one side as a phenomenon secondary to coagulation. With the discovery of TAFI as an inhibitor of fibrinolysis in 1995, interest in the role of the fibrinolytic system was reignited. Increased TAFI levels appeared to increase the risk of a first or recurrent venous thrombosis in several studies.[20–22] Studies on the association between single-nucleotide polymorphisms (SNPs) that are associated with TAFI levels and the risk of venous thrombosis produced conflicting results.[23–28]

As the ECLT and DWBCLT have some limitations, an assay assessing the overall fibrinolytic potential in plasma has recently been developed. The ECLT measures the lytic activity in the euglobulin fraction of plasma, and mainly reflects the balance between plasminogen activators and PAIs on plasminogen activation. The DWBCLT measures the lytic activity on fibrin of plasmin in the presence of citrate and excludes the downregulation of
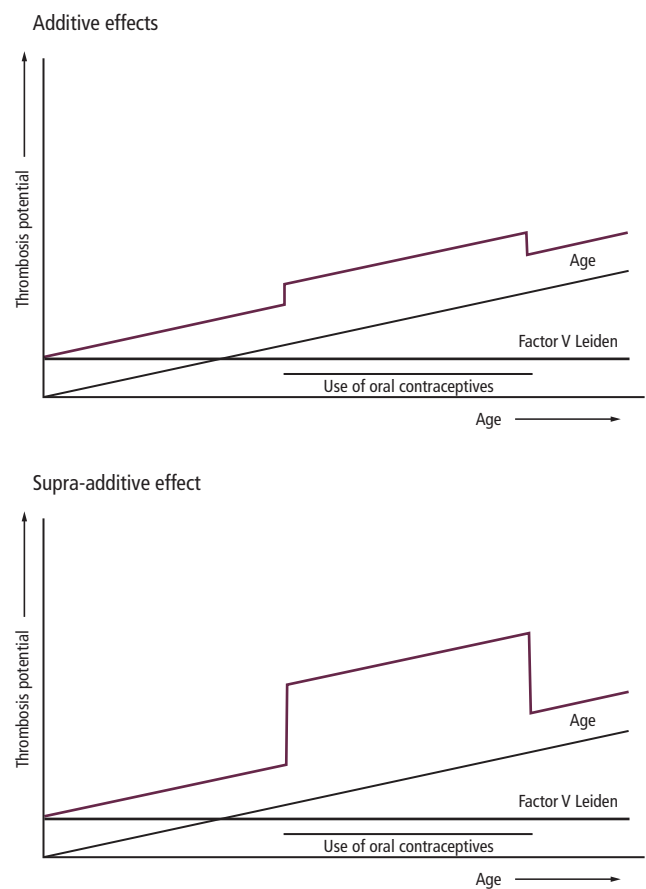
fibrinolysis via thrombin, factor XIII and TAFI. In the new assay, clotting of the blood is induced by tissue factor and lysis is initiated by adding exogenous t-PA. The clot lysis potential as determined by this assay not only is determined by PAI-1, α_2-antiplasmin and plasminogen, but also is influenced by levels of TAFI and by the interplay between coagulation and TAFI activation.[29] The assay is probably not sensitive for variations in plasma levels of t-PA, as clot lysis is induced in the assay with a fixed amount of exogenous t-PA. Plasminogen seems to influence the CLT only when levels are below 40% of pooled normal plasma.

The assay was first used to assess the relationship between hypo-fibrinolysis and venous thrombosis in the Leiden Thrombophilia Study (LETS). This was a population-based case-control study with 474 patients and 474 control subjects.[30] A dose–response relationship between CLT and the risk of venous thrombosis was found when using the 70th, 80th, 90th, 95th and 99th percentiles and based on values found in control subjects at cut-off points. Odds ratios (ORs) of 1.4, 1.6, 1.9, 2.1 and 2.2 were found, respectively, for those with CLT over the cut-off compared with individuals with CLT below this point. While 10% of the controls had CLT above the 90th percentile, 18% of the patients had CLT over this cut-off, resulting in a two-fold increased risk of venous thrombosis for individuals with CLT above the 90th percentile.

Confirmation of this association was demonstrated by the measurement of CLT in the Multiple Environmental and Genetic Assessment (MEGA) of risk factors for venous thrombosis.[31] This case-control study included almost 2,500 patients between 17 and 80 years of age and 3,000

controls. It was designed to study the risk of venous thrombosis in individuals with multiple risk factors. A clear dose-dependent increase in the risk of venous thrombosis was found in individuals with hypo-fibrinolysis. Using deciles of CLT, a three-fold increase in risk was found for those who had CLTs over the 90th percentile compared with those below the 10th percentile. The risk was similar in men and women, the younger and older subjects and for both deep vein thrombosis of the leg and pulmonary embolism. It was further hypothesised that decreased fibrinolytic potential in combination with risk factors causing hypercoagulability would result in synergistic effects on the risk of venous thrombosis. ORs were calculated for the joint presence of hypo-fibrinolysis and the prothrombin 20210A mutation (OR 4.4), factor V Leiden (OR 8.1) and immobilisation (surgery, bed rest or plaster cast) (OR 10.3). The strongest effect was seen in women below 50 years of age who used oral contraceptives and had prolonged CLTs. Women who have CLT above the 75th percentile (4th quartile) but do not take oral contraceptives have a risk that is almost two-fold higher than that of women who have CLT below the 25th percentile (1st quartile) and do not take oral contraceptives (OR 1.9, 95% confidence interval [CI] 1.1–3.3). Women who take oral contraceptives and have short CLT (below the 25th percentile) have an OR of 2.6 (95% CI 1.6–4.0) compared with women with similar CLT not taking oral contraceptives. In women who have both risk factors – CLT above the 75th percentile and the use of oral contraceptives – a more than 20-fold increased risk of venous thrombosis (OR 21.8, 95% CI 10.2–46.7) was observed. The expected OR based on an additive effect of the individual risks was only 3.5. Incorporating these results in the model of thrombosis risk leads to what is shown in *Figure 2B*, where the horizontal line describing the risk associated with factor V Leiden is replaced by a horizontal line depicting the risk associated with hypofibrinolysis. The other lines remain the same.

The determinants of the CLT test are not completely known. Current research aims to elucidate the genetic and environmental factors influencing CLT. The strongest determinant of CLT seems to be the plasma level of PAI-1, but levels of other fibrinolytic factors also appear to play a role.

A strong association was observed between CLT and body mass index (BMI). This could be explained by the increase in PAI-1 levels with an increasing BMI.[32]

Figure 3: Simplified Representation of the Fibrinolytic System
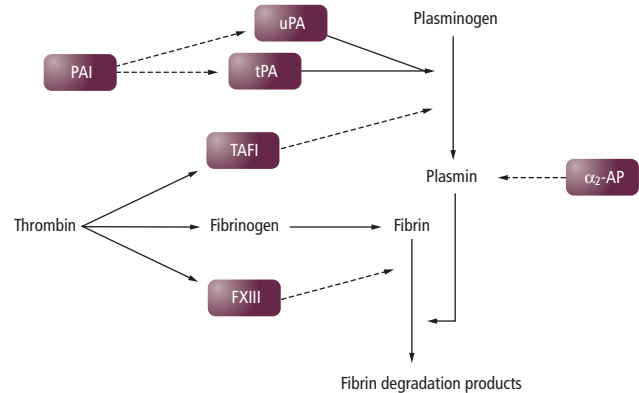
Continuous arrows denote stimulation and activation. Interrupted arrows denote inhibition.

The results of the MEGA study on BMI and venous thrombosis[33] showed strong parallels with our study on hypofibrinolysis and venous thrombosis. The combined effect of obesity and oral contraceptive use showed effects on thrombosis risk similar to the combination of hypofibrinolysis and oral contraceptive use. An OR of 23.8 (95% CI 13.4–42.3) was found in obese (BMI ≥30) women taking oral contraceptives compared with women with a BMI <25 who were not taking oral contraceptives. Obesity alone is only a mild risk factor (OR 3.0, 95% CI 1.7–5.6). Although obesity influences several haemostatic markers, it may be that the joint effect of the combination of oral contraceptives with obesity works partly via the same mechanism as the combination of oral contraceptives with an increased CLT (i.e. increased PAI-1 levels).

Conclusion

A hypofibrinolytic state, as measured by an overall fibrinolytic plasma-based assay, has been shown to be associated with an increased risk of venous thrombosis in two large independent case-control studies. Those individuals who have both an increased coagulation potential, caused by certain genetic or environmental factors, and a decreased fibrinolytic potential are at a higher risk than would be expected from the sum of the individual risks. As plasminogen itself does not appear to be associated with thrombotic risk, these results seem contradictory, and further research is necessary to improve our understanding of the underlying mechanism. ∎

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