

## Levels of coagulation factors and venous thromboembolism

ARMANDO TRIPODI

**Background and Objectives.** Altered levels of coagulation factors have been included among the abnormalities that may increase the risk of venous thromboembolism (VTE) in otherwise healthy subjects.

**Information Sources.** According to the studies that have been carried to test this hypothesis only elevated levels of factor VIII and fibrinogen emerged as independent risk factors for VTE.

**State of the Art and Perspectives.** Although some data indicate that elevated levels of factor XI or IX are also determinants for VTE, this awaits confirmation.

**Key words:** coagulation factors, thrombosis, laboratory investigation.

Haematologica 2003; 88:705-711  
[http://www.haematologica.org/2003\\_06/705.htm](http://www.haematologica.org/2003_06/705.htm)

©2003, Ferrata Storti Foundation

The maintenance of blood fluidity within the vascular system is ensured by the balance between pro-coagulant and anticoagulant forces, which operate in plasma as well as in circulating and stationary cells. The rapid expansion of knowledge during the last decades has unraveled new mechanisms that regulate thrombogenesis, and these have been successfully exploited to investigate patients at increased risk of developing venous thromboembolism (VTE). Many conditions have been recognized to increase the risk of VTE. The most frequent of these are congenital deficiencies or abnormalities of naturally occurring anticoagulants (antithrombin and protein C/S), the presence of common polymorphisms in the genes of coagulation factors (factor V, prothrombin) and acquired conditions such as the antiphospholipid antibody syndrome and moderate hyperhomocysteinemia.<sup>1</sup> More recently, it was postulated that even increased levels of procoagulant factors may constitute a risk factor for VTE and this paved the way to a number of studies designed to show whether

the biological plausibility which is behind this concept is worthy of exploration. The aim of this article is to review the literature with respect to this issue and give guidance to clinicians and laboratory workers on whether and, if so, how they should embark on such investigations for thrombophilic patients.

### Coagulation factors of the contact system

Although the coagulation factors involved in the contact system are known to help initiate coagulation *in vitro* (Figure 1), their role *in vivo* has been negated by the evidence that, with the notable exception of factor XI, congenital deficiency of these molecules (pre-kallikrein, high molecular weight kininogen and factor XII) is not associated with clinical bleeding. However, the link between the contact system and the activation of fibrinolysis made it plausible to surmise that low levels might lead to hypofibrinolysis and hence increase the risk of thrombosis. Among the contact factors, factor XII has been extensively investigated in numerous studies, whereas all the others, except factor XI, have received very little attention.

### Factor XII

Lammler *et al.* performed a retrospective analysis of 18 patients with homozygous factor XII deficiency, 45 patients with heterozygous factor XII deficiency and 11 non-deficient subjects.<sup>2</sup> They recorded no bleeding events; there were 2 episodes of VTE in patients with homozygous deficiency and none in patients with heterozygous deficiency. Their conclusion of a possible association of factor XII deficiency and VTE is hampered by the retrospective nature of their observation and by the small numbers of patients investigated. Von Kanel *et al.* measured factor XII levels in 200 patients with idiopathic VTE and in 200 healthy controls.<sup>3</sup> They found no difference (activity or antigen) between cases and controls.<sup>3</sup> Zeerleder *et al.* investigated 15 severely- and 35 partially-factor XII-deficient subjects together with 11 healthy subjects.<sup>4</sup> Analysis of the Kaplan-Meier thrombosis-free survival curves suggested that partial (and probably severe) factor XII deficiency did not constitute a risk factor for VTE.<sup>4</sup> Apparently, this was a re-evaluation of the studies reported by Lammler *et al.*<sup>2</sup> and by von Kanel *et al.*<sup>3</sup> Halbmayer *et al.* investigated 103 patients who were on oral anticoagulants because of a previous episode of VTE, or arterial thrombosis and 50 healthy subjects.<sup>5</sup> They found that 8% of factor XII-deficient patients had VTE and 20% had arterial thrombosis.<sup>5</sup> They concluded that

From the Angelo Bianchi Bonomi, Hemophilia and Thrombosis Center, Department of Internal Medicine, University and IRCCS Maggiore Hospital, Milan, Italy. This peer-reviewed survey has been prepared upon invitation of the Council of the Italian Society for Hemostasis and Thrombosis (SISET).

Correspondence: Armando Tripodi, MD, Angelo Bianchi Bonomi, Hemophilia and Thrombosis Center, Department of Internal Medicine, University and IRCCS Maggiore Hospital, via Pace 9, 20122 Milan, Italy.  
E-mail: [armando.tripodi@unimi.it](mailto:armando.tripodi@unimi.it)

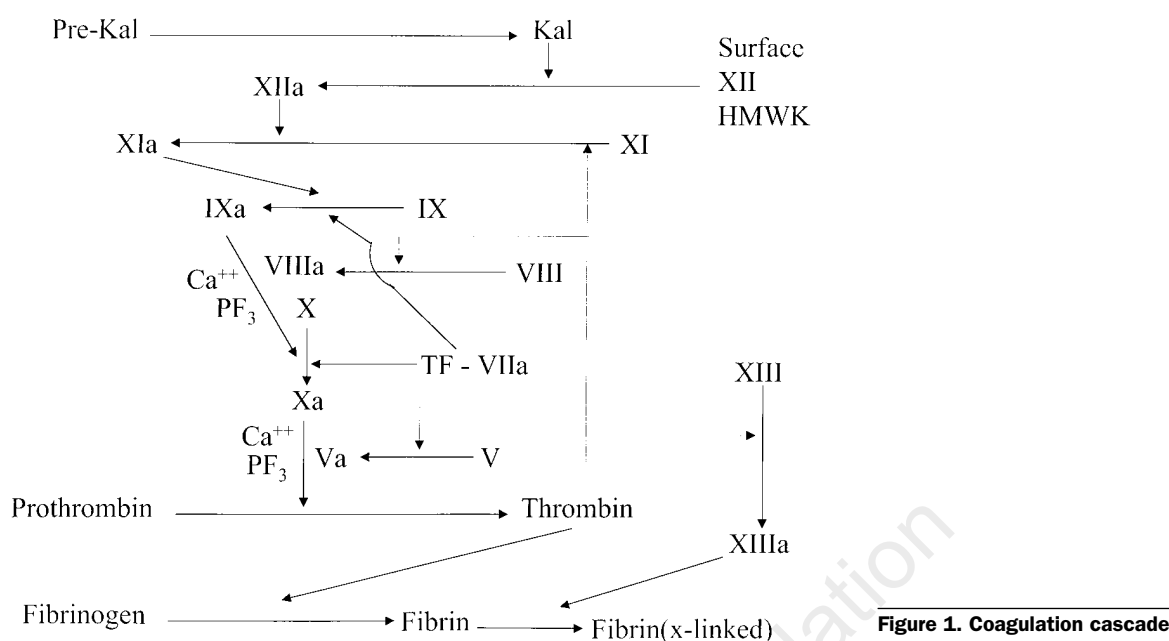


Figure 1. Coagulation cascade.

low levels of factor XII should be considered as a risk factor for thromboembolism.<sup>5</sup> Koster *et al.* included factor XII measurements in their population-based case-control study for thrombophilia (the Leiden Thrombophilia Study, LETS). The authors enrolled consecutive patients ( $n=350$ ) with at least one episode of documented VTE and a population of control subjects ( $n=350$ ) matched for sex, age and living conditions to the population of patients.<sup>6</sup> The frequency of moderate factor XII deficiency (<57%) recorded for cases (6%) was not different from that recorded for controls (5%). The odds ratio for venous thrombosis was 1.6 (95% confidence interval, 0.6-2.4).<sup>6</sup> On the basis of the above studies one should conclusively reject the role of low levels of factor XII in VTE.

### Factor XI

In the past, factor XI was thought to be activated mainly by activated factor XII. More recently, it was shown that the physiologic activation is mediated by thrombin in conjunction with or without negatively charged cellular surfaces.<sup>7,8</sup> (Figure 1). Elevated levels of factor XI have been surmised to be associated with thrombosis. The likely mechanism is excessive thrombin generation, which may lead to excessive deposition of the thrombin and downregulation of fibrinolysis through the activation of the thrombin activatable fibrinolysis inhibitor (TAFI).<sup>9</sup> Interestingly, elevated levels of TAFI emerged as an independent risk factor for VTE in the LETS study.<sup>10</sup> Indirect evidence for the association of elevated factor XI levels and thrombosis stems from the occur-

rence of activation of the coagulation cascade after infusion of factor XI concentrates in congenitally deficient patients.<sup>11,12</sup> However, the most compelling evidence comes from the LETS study. The authors measured factor XI antigen in plasmas from 474 unselected patients with a first, objectively confirmed episode of deep vein thrombosis and in plasmas from an equal number of healthy controls.<sup>13</sup> The odds ratio for deep vein thrombosis (DVT) for patients with factor XI levels above the 90th percentile of the distribution of controls (121%) was 2.2 (95% confidence interval, 1.5-3.2).<sup>13</sup> Adjustment for other congenital or acquired risk factors for VTE did not alter the results.<sup>13</sup> Should these findings be confirmed by other studies, one should accept that high levels of factor XI play a role in VTE.

### Factor IX

Activated factor IX is a key enzyme in the coagulation cascade, serving as one of the activators for factor X (Figure 1). Its importance for hemostasis *in vivo* is clearly documented by the severity of hemophilia B secondary to its congenital deficiency. Because of this, it was surmised that elevated levels could be associated with VTE. To test this hypothesis van Hylekama *et al.* included factor IX in the LETS study and measured the antigen concentration in plasmas from 426 patients with a first, objectively proven episode of DVT and in plasma from 473 healthy controls.<sup>14</sup> The odds ratio for DVT for patients with factor IX levels above the 90th percentile of the distribution of healthy controls (129%) compared to those below this level was 2.3

(95% confidence interval, 1.6-3.5).<sup>14</sup> Adjustment for other congenital or acquired risk factors did not alter the results.<sup>14</sup> Should these findings be confirmed by other studies, one should accept that high levels of factor IX play a role in VTE.

### Factor VIII

Activated factor VIII plays a crucial role as a co-factor in the activation of factor X mediated by activated factor IX (Figure 1). The importance of factor VIII in maintaining the integrity of the hemostatic system parallels that of factor IX and is documented by the severity of hemophilia A, which is secondary to congenital deficiency of factor VIII. Early observations had shown that increased levels of factor VIII were able to shorten considerably the *in vitro* clotting time measured by the activated partial thromboplastin time (APTT), but it was not until recently that the interplay between elevated factor VIII levels and venous thrombosis was documented. Indirect evidence for this interplay comes from a new animal model of thrombophilia showing that high plasma levels of factor VIII are indeed thrombogenic.<sup>15</sup> Furthermore, marked elevation of thrombin generation has been found in patients with elevated levels of factor VIII and VTE.<sup>16</sup> In 1995 Koster *et al.* measured factor VIII clotting activity as part of the LETS study in plasma from 301 patients and 301 controls.<sup>17</sup> The adjusted odds ratio for DVT in patients with factor VIII levels above 150% compared to those with levels below 100% was 4.8 (95% confidence interval, 2.3-10.0).<sup>17</sup> These findings were later confirmed and extended by Kraaijenhagen *et al.* who investigated 65 patients with a proven single episode of VTE, 60 patients with proven recurrent VTE and 60 patients with suspected, but not confirmed VTE.<sup>18</sup> It was found that 19% of patients who had had a single episode of VTE and 33% of patients who had had recurrent episodes had factor VIII levels above the 90<sup>th</sup> percentile of distribution of the control population (175%).<sup>18</sup> Furthermore, for each 10% increase in factor VIII, the risk for a single and recurrent episode of venous thrombosis increased by 10% (95% confidence interval, 0.9-21.0) and 24% (95% confidence interval, 1.1-38.0), respectively.<sup>18</sup> More recently, Kyrle *et al.* investigated the value of elevated factor VIII levels as a risk factor for recurrent VTE.<sup>19</sup> They prospectively investigated 360 patients, the average follow-up being 30 months after the first episode of VTE and discontinuation of oral anticoagulation. Patients with recurrences had mean factor VIII levels higher (182±66) than those without (157±54).<sup>19</sup> The adjusted relative risk for recurrence in patient with factor VIII levels above the 90<sup>th</sup> percentile was 6.7 (95% confidence interval, 3.0-14.8).<sup>19</sup> In conclusion, there is now convincing evidence that elevated factor VIII is a risk factor for recurrent VTE.

Since factor VIII is a well-known acute phase

**Table 1. Coagulation factor levels associated with venous thromboembolism.**

Coagulation factor	Odds ratio (95% C.I.)	Reference
Factor XII (< 57 %)	1.2 (0.6-2.4)	6
Factor XI (> 121%)	2.2 (1.5-3.2)	13
Factor IX (> 129%)	2.3 (1.6-3.5)	14
Factor VIII (> 150%)	4.8 (2.3-10.0)	17-21
Factor X (> 126%)	1.6 (1.1-2.4) <sup>a</sup>	30
Factor V (> 150%)	1.3 (0.9-1.8)	29
Factor V (upper quintile)	11.5 (4.2-31.4) <sup>b</sup>	32
Factor II (> 115%)	2.1 (1.5-3.1)	33
Factor II (> 108%)	1.9 (1.1-3.2)	35
Fibrinogen (> 500 mg/dL)	4.3 (1.7-10.5)	21
Factor VII (> 110%)	0.8 (0.4-1.5)	40
Factor XIII	N.D.	

<sup>a</sup>The odds ratio was no longer significant after adjustment for other vitamin K-dependent coagulation factors.

<sup>b</sup>The odds ratio refers to the combination of elevated factor V levels and factor V Leiden.

reactant, O' Donnell and co-workers investigated the role of increased factor VIII synthesis and the relationship with the acute phase reaction.<sup>20</sup> To this end the authors studied 260 consecutive patients who were referred for thrombophilia screening. Twenty-five percent had elevated levels (>150%) of factor VIII and the factor VIII levels were not correlated with the levels of C reactive protein,<sup>20</sup> which is a well-known acute phase reactant. Kamphuisen and co-workers, in a reappraisal of the LETS study,<sup>21</sup> drew a similar conclusion. After adjustment for C reactive protein, high factor VIII levels increased the risk of thrombosis by 6-fold.<sup>21</sup>

A question of practical interest concerns the heritability of elevated factor VIII levels.<sup>22-24</sup> Kamphuisen and co-workers investigated this issue as part of the LETS study<sup>23</sup> by analysis of the familial influence of factor VIII levels (>150%) in 12 families with thrombophilia. It emerged that blood group was the main determinant of factor VIII levels (blood group O, lower factor VIII levels than blood group non-O). Furthermore, familial clustering of factor VIII levels (>150%) was shown by the familial aggregation test and this remained after adjustment for the effect of blood group and age.<sup>23</sup> Additional studies on factor VIII heritability were carried out by Schambeck and co-workers who also reported familial clustering of high factor VIII levels in patients with VTE.<sup>24</sup> However, Mansvelt and co-workers and Kamphuisen and co-workers failed to identify any polymorphism in the promoter gene of

factor VIII,<sup>25</sup> or in the genes of factor VIII and von Willebrand factor<sup>26</sup> responsible for the heritability in individuals with high factor VIII levels and venous thrombosis.

A further question of practical importance concerns how to measure factor VIII. Presently there are three types of methods available: (i) the clotting activity assay, which measures factor VIII clotting activity through the APTT-based method and factor VIII-deficient plasma;<sup>27</sup> (ii) the ELISA, which measures factor VIII antigen using two monoclonal antibodies directed against the light chain;<sup>26</sup> (iii) the amidolytic method, which measures the amount of thrombin generated by plasma factor VIII in a purified system through a synthetic chromogenic substrate.<sup>28</sup> These three methods have been used in different studies on the epidemiology of factor VIII.<sup>17,29,24</sup> Their results should be regarded as equivalent.

### **Factor X**

Activated factor X represents the active enzyme of the tenase complex and is able to convert prothrombin to thrombin (Figure 1). The reaction is mediated by activated factor V and takes place on anionic phospholipids. De Visser and co-workers included factor X measurement in their LETS study by investigating 474 patients and 474 controls.<sup>30</sup> The odds ratio for VTE in patients with factor X levels above the 90th percentile (126%) was barely significant (1.6; 95% confidence interval, 1.1-2.4)<sup>31</sup> and, indeed, was no longer significant after adjustment for the levels of the other vitamin K dependent coagulation factors,<sup>30</sup> indicating that the risk is probably mediated by other factors. Based on the present limited evidence one should conclude that the levels of factor X do not play a role in VTE.

### **Factor V**

Activated factor V is homologous to activated factor VIII in many respects. Both are activated by thrombin; both are inhibited by activated protein C and both act as co-factors in two crucial steps of the coagulation cascade (Figure 1). Because of these similarities it was surmised that even high levels of factor V could be considered as a putative candidate risk factor for VTE. Moreover, factor V acts in synergy with protein S as the co-factor for activated protein C in the inhibition of factor VIII.<sup>31</sup> Hence, even low factor V could play a role in thrombogenesis. Kamphuisen and co-workers included the measurement of factor V in the LETS study and investigated 474 patients and 474 controls.<sup>29</sup> Neither low nor high levels of factor V were associated with VTE.<sup>29</sup> Recently, Folsom and co-workers found that increased levels of factor V were a risk factor for venous thrombosis, but only in combination with factor V Leiden (Odds ratio, 11.5; 95% confidence interval, 4.2-31.4).<sup>32</sup> Based on the present limited

evidence one should conclude that the levels of factor V do not play a role in VTE.

### **Factor II**

Factor II (or prothrombin) is the precursor of thrombin, the key enzyme in the coagulation cascade (Figure 1). Its involvement in thrombogenesis, though predictable, had never been documented until 1996, when Poort and co-workers described a polymorphism in the 3' untranslated region of the prothrombin gene, which involves the transition G→A at position 20210.<sup>33</sup> For reasons that have been subsequently clarified,<sup>34</sup> this polymorphism results in a phenotype characterized by moderate hyperprothrombinemia.<sup>33</sup> Within the frame of the LETS study, Poort and co-workers investigated this polymorphism and prothrombinemia in 474 unselected patients with a first, objectively confirmed episode of DVT and in 474 controls. Carriers of the polymorphism had a higher risk of developing thrombosis than did non-carriers.<sup>33</sup> Interestingly, subjects with prothrombin levels greater than 115% (90th percentile) also had a 2.1-fold increased risk (95% confidence interval, 1.5-3.1) of VTE.<sup>33</sup> Hyperprothrombinemia was also associated albeit weakly with VTE (odds ratio, 1.9; 95% confidence interval, 1.1-3.2) in a study by Cattaneo *et al.* of 118 patients and 416 controls.<sup>35</sup> However, because of the wide distribution of prothrombin levels in carriers and non-carriers of the polymorphism, prothrombin measurement alone is not suitable for distinguishing carriers from non-carriers.<sup>36,37</sup>

### **Fibrinogen**

Various studies have clearly shown that a high level of fibrinogen is a good predictor of arterial thrombosis in otherwise healthy subjects.<sup>38,39</sup> Koster and co-workers were among the first to investigate the association between high fibrinogen and the risk of VTE.<sup>40</sup> As part of the LETS study they investigated 199 patients and 199 controls. The odds ratio for VTE in subjects with a plasma fibrinogen level exceeding 500 mg/dL was 3.7 (95% confidence interval, 0.7-19.0).<sup>40</sup> Although the odds ratio was well above unity, the limited number of subjects investigated resulted in a wide confidence interval. A subsequent re-evaluation of the same issue was attempted by Kamphuisen and co-workers who increased the number of subjects belonging to the same cohort of patients.<sup>21</sup> They investigated 474 patients and 474 controls. The adjusted odds ratio for VTE in subjects with a plasma fibrinogen level exceeding 500 mg/dL was 4.3 (95% confidence interval, 1.7-10.5).<sup>21</sup> Furthermore, they concluded that the increased levels were not due to the acute phase reaction.<sup>21</sup> In conclusion, the evidence suggests that high fibrinogen is a risk factor for VTE. Although numerous different methods are available, the above studies measured the fibrinogen concen-

tration according to the von Clauss (coagulation) method.

### **Factor VII**

Factor VII, in combination with its cell receptor tissue factor, plays a pivotal role in the initiation of coagulation *in vivo* (Figure 1). Earlier epidemiological studies showed that moderately high factor VII levels are a good predictor of arterial thrombosis.<sup>38</sup> Subsequent studies failed to confirm these findings.<sup>41,42</sup> This discrepancy may be attributed to the different properties of the thromboplastins used for testing factor VII in different studies. The involvement of factor VII in venous thrombosis was tested by Koster and co-workers in the LETS study.<sup>40</sup> They investigated 199 patients and 199 controls. The results were disappointing. The odds ratio for VTE in subjects with plasma factor VII levels greater than 110% (90th percentile) was 0.8 (95% confidence interval, 0.4–1.5) (40). The limits of confidence were narrow and this suggests that increasing the number of subjects would not affect the conclusions.

Based on the present limited evidence one should conclude that the levels of factor VII do not play a role in VTE.

### **Factor XIII**

Activated factor XIII catalyzes the covalent cross-linking of  $\alpha$ - and  $\gamma$ -fibrin chains, thus stabilizing the fibrin clot and increasing its resistance to fibrinolysis (Figure 1). Its role in hemostasis is well documented by the rare, but severe hemorrhagic diathesis that occurs in patients with deficiency of this factor. Although factor XIII levels have never been directly investigated for their association with VTE, the factor XIII Val34Leu polymorphism of the A sub-unit, apparently associated with enhanced activation of zymogenic factor XIII,<sup>43</sup> was recently identified as a protective genetic factor for arterial<sup>44</sup> and venous<sup>45</sup> thrombosis. The latter association has, however, been disputed.<sup>46,47</sup> Furthermore, the relationship between factor XIII levels as measured either by activity or antigen and this polymorphism remains unclear. In conclusion, the present limited evidence is not such to suggest that factor XIII levels play a role in VTE.

### **Conclusive remarks and perspectives**

Evidence accumulated during the last few years and especially that from the well-designed, population-based case-control LETS study, is such to substantiate the biologically plausible concept that an imbalance in favor of procoagulant factors may be considered as a possible triggering factor for VTE. Even though not all coagulation factors have emerged as risk factors for VTE, some of them (factor VIII and fibrinogen) are now well established to be so (Table 1). This does not necessarily mean that they should be included in the laboratory work-up to

investigate thrombophilic patients. As a matter of fact, it should be recognized that the value of laboratory investigations of thrombophilia is currently being debated and there is no consensus on whether or not other well established genetic conditions leading to thrombophilia should be investigated.<sup>48,49</sup> The argument against is that the investigation does not affect the management of patients with thrombosis.<sup>48</sup> The arguments in favor are that the investigation may be of help in preventing recurrences in patients with previous events, or in preventing events in asymptomatic family members of probands.<sup>49</sup> Furthermore, an additional argument in favor is that the laboratory investigation (if comprehensive) may help to identify patients bearing combined defects who are at increased risk of developing thrombosis. In this respect inclusion of factor VIII and fibrinogen in the laboratory work-up may help to define the risk profile in individual patients, especially if they carry other prothrombotic mutations, or plasma abnormalities known to trigger thrombosis.<sup>50,51</sup> Therefore, it should be concluded that their inclusion in the thrombophilia work-up is beneficial.

On the other hand, it should be realized that thrombophilia has contributed significantly to increase the pressure on clinical laboratories and demands for testing are rising dramatically. The cost of testing for thrombophilia is also increasing, as the current strategy is to perform individual measurements rather than screening patients with global tests.<sup>1</sup> Ideally, global tests should be responsive to most of the prothrombotic factors (including the defects of naturally-occurring anticoagulant mechanisms), but these tests are not yet available.

In principle, high levels of coagulation factors could be detected by global tests such as the APTT, which is sensitive to most of them. Although, no data are presently available it can be expected that this strategy would be of limited value.

The time-honored thrombin generation test<sup>52</sup> could be a suitable alternative. This test has been recently revisited by Hemker and Beguin and adapted to measure endogenous thrombin potential after activation of plasma coagulation with tissue factor or cephaline.<sup>53</sup> Kyrle and co-workers employed this test to show increased thrombin generation in plasma from patients with hyperprothrombinemia.<sup>54</sup>

Activated protein C (APC) resistance, defined as a defective prolongation of the plasma clotting time upon addition of APC,<sup>55</sup> could be another alternative. The basic test described by Dahlback and co-workers<sup>55</sup> has been found to be responsive to increased levels of factor VIII<sup>56,57</sup> or prothrombin.<sup>58</sup> This suggests that an imbalance of the coagulation cascade in favor of procoagulant factors may cause an acquired resistance to APC. Since acquired APC resistance (not due to factor V mutations) has recently been described to be an independent risk factor for VTE<sup>56,59</sup> it could be surmised that sensitive

assays for APC resistance are able to detect the procoagulant imbalance leading to thrombosis. A potential candidate to be explored is the APC resistance test based on thrombin generation, recently described by Rosing *et al.*<sup>60</sup> The fact that this test was able to detect the subtle difference of acquired APC resistance secondary to oral contraceptive (third- versus second-generation) intake<sup>60,61</sup> is very promising and deserves further investigation.

## References

1. Tripodi A, Mannucci PM. Laboratory investigation of thrombophilia. *Clin Chem* 2001;47:1597-606.
2. Lammler B, Wuillemin WA, Huber I, Krauskopf M, Zurcher C, Pflugschaupt R, et al. Thromboembolism and bleeding tendency in congenital factor XII deficiency. A study on 74 subjects from 14 Swiss families. *Thromb Haemost* 1991;65:117-21.
3. Von Kanel R, Wuillemin WA, Furlan M, Lammler B. Factor XII clotting activity and antigen levels in patients with thrombotic disease. *Blood Coag Fibrinol* 1992;3:555-61.
4. Zeerleder S, Schloesser M, Redondo M, Wuillemin WA, Engel W, Furlan M, et al. Reevaluation of the incidence of thrombotic complications in congenital factor XII deficiency. A study on 73 subjects from 14 Swiss families. *Thromb Haemost* 1999; 82:1240-6.
5. Halbmayer WM, Mannhalter C, Feichtinger C, Rubi K, Fischer M. The prevalence of factor XII deficiency in 103 orally anticoagulated outpatients suffering from recurrent venous and/or arterial thromboembolism. *Thromb Haemost* 1992; 68:285-90.
6. Koster T, Rosendaal FR, Briet E, Vandenbroucke JP. John Hageman's factor and deep-vein thrombosis. Leiden thrombophilia study. *Brit J Haematol* 1994;87:422-4.
7. Naito K, Fujikawa K. Activation of human blood coagulation factor XI independent of factor XII: factor XI is activated by thrombin and factor XIa in the presence of negatively charged surfaces. *J Biol Chem* 1991;266:7353-8.
8. Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. *Science* 1991;253:909-12.
9. Von dem Borne PA, Bajar L, Meijers JC, Nesheim ME, Bouma BN. Thrombin mediated activation of factor XI results in a thrombin-activatable fibrinolysis inhibitor-dependent inhibition of fibrinolysis. *J Clin Invest* 1997;99:2323-7.
10. Van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. *Blood* 2000;95:2855-9.
11. Mannucci PM, Bauer KA, Santagostino E, Faioni E, Barzegar S, Coppola R, et al. Activation of the coagulation cascade after infusion of a purified concentrate in congenitally deficient patients. *Blood* 1994;84:1314-9.
12. Richards EM, Makris MM, Cooper P, Preston FE. In vivo coagulation activation following infusion of highly purified factor XI concentrate. *Br J Haematol* 1997;96:293-7.
13. Meijers JCM, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of factor XI as a risk factor for venous thrombosis. *N Engl J Med* 2000;342:696-701.
14. Van Hylckama A, van der Linden IK, Bertina RM, Rosendaal FR. High levels of factor IX increase the risk of venous thrombosis. *Blood* 2000;95:3678-82.
15. Kawasaki T, Kaida T, Arnout J, Vermeylen J, Hoylaerts MF. A new animal model of thrombophilia confirms that high plasma levels of factor VIII levels are thrombogenic. *Thromb Haemost* 1999;81:306-11.
16. O'Donnell J, Mumford AD, Manning RA, Laffan MA. Marked elevation of thrombin generation in patients with elevated FVIII:C and venous thromboembolism. *Br J Haematol* 2001;115:687-91.
17. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep vein thrombosis. *Lancet* 1995;345:152-5.
18. Kraaijenhagen RA, Anker PS, Koopman MM, Reitsma PH, Prins MH, van den Ende A, et al. High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. *Thromb Haemost* 2000;83:5-9.
19. Kyrle PA, Minar E, Hirschl M, Bialonczyk C, Stain M, Schneider B, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. *N Engl J Med* 2000;343: 457-62.
20. O'Donnell J, Mumford AD, Manning RA, Laffan M. Elevation of FVIII:C in venous thromboembolism is persistent and independent of the acute phase response. *Thromb Haemost* 2000;83:10-3.
21. Kamphuisen PW, Jeroen C, Eikenboom J, Vos HL, Pablo R, Sturk A, Bertina RM, et al. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. *Thromb Haemost* 1999;81:680-3.
22. Kamphuisen PW, Houwing-Duistermaat JJ, van Houwelingen HC, Eikenboom JC, Bertina RM, Rosendaal FR. Familial clustering of factor VIII and von Willebrand factor levels. *Thromb Haemost* 1998;79:323-7.
23. Kamphuisen PW, Lensen R, Houwing-Duistermaat JJ, Eikenboom JC, Harvey M, Bertina RM, et al. Heritability of elevated factor VIII antigen levels in factor V Leiden families with thrombophilia. *Br J Haematol* 2000;109:519-22.
24. Schambeck CM, Hinney K, Haubitz I, Mansouri-Talegani B, Wahler D, Keller F. Familial clustering of high factor VIII levels in patients with venous thromboembolism. *Arterioscler Thromb Vasc Biol* 2001;21:289-92.
25. Mansvelt EP, Laffan M, McVey JH, Tuddenham EG. Analysis of the F8 gene in individuals with high plasma factor VIII:C levels and associated thrombosis. *Thromb Haemost* 1998;80: 561-5.
26. Kamphuisen PW, Eikenboom JC, Rosendaal FR, Koster T, Blann AD, Vos HL, et al. High factor VIII antigen levels increase the risk of venous thrombosis but are not associated with polymorphisms in the von Willebrand factor and factor VIII gene. *Br J Haematol* 2001;115:156-8.
27. Mannucci PM, Tripodi A. Factor VIII clotting activity. In: J Jespersen, RM Bertina, F Haverkate, Editors. *Laboratory techniques in thrombosis. A manual.* Kluwer Academic Publishers, Dordrecht 1999; p. 107-13.
28. Tripodi A, Mannucci PM. Factor VIII activity as measured by an amidolytic assay compared with one-stage clotting assay. *Am J Clin Plathol* 1986;86:341-4.
29. Kamphuisen PW, Rosendaal FR, Eikenboom JC, Bos R, Bertina RM. Factor V antigen levels and venous thrombosis. Risk profile, interaction with factor V Leiden and relation with factor VIII antigen levels. *Arterioscler Thromb Vasc Biol* 2000;20:1382-6.
30. De Visser MC, Poort SR, Vos HL, Rosendaal FR, Bertina RM. Factor X levels, polymorphisms in the promoter region of factor X and the risk of venous thrombosis. *Thromb Haemost* 2001;85:1011-7.
31. Shen L, Dahlback B. Factor V and protein S as synergistic cofactors to activated protein C in degradation of factor VIIa. *J Biol Chem* 1994;269:18735-8.
32. Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, Boland LL, et al. A prospective study of venous thromboembolism in relation to factor V Leiden and related factors. *Blood* 2002;99:2720-5.
33. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and increase in venous thrombosis. *Blood* 1996;88:3698-703.
34. Soria JM, Almasy L, Souto JC, Tirado I, Borrell M, Mateo J, et al. Linkage analysis demonstrates that prothrombin G20210A mutation jointly influences plasma prothrombin levels and risk of thrombosis. *Blood* 2000;95:2780-5.
35. Cattaneo M, Chantarangkul V, Taioli M, Santos Hermida J, Tagliabue L. The G20210A mutation of the prothrombin gene in patients with previous first episodes of deep-vein thrombosis: prevalence and association with factor V G1691A, methylenetetrahydrofolate reductase C677T and plasma prothrombin levels. *Thromb Res* 1999;93:1-8.
36. Makris M, Preston FE, Beauchamp NJ, Cooper PC, Daly ME,

- Hampton KK, et al. Co-inheritance of the 20210A allele of the prothrombin gene increases the risk of thrombosis in subjects with familial thrombophilia. *Thromb Haemost* 1997;78:1426-9.
37. Grunewald M, Germowitz A, Beneke H, Guethner C, Griesshammer M. Coagulation factor II activity determination is not useful as a screening tool for the G20210A prothrombin gene allele. *Thromb Haemost* 2000;84:141-2.
  38. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, et al. Haemostatic function and ischemic heart disease. Principal results of the Northwick Park Heart Study. *Lancet* 1986;2:533-7.
  39. Heinrich J, Balleisen L, Schulte H, Assmann G, van de Loo J. Fibrinogen and factor VII in the prediction of coronary risk. *Arterioscler Thromb Vasc Biol* 1994;14:54-9.
  40. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case control study of plasma levels and DNA polymorphism. The Leiden thrombophilia study (LETS). *Thromb Haemost* 1994;71:719-22.
  41. Junker R, Heinrich J, Schulte H, van de Loo J, Assman G. Coagulation factor VII and the risk of coronary heart disease in healthy men. *Arterioscler Thromb Vasc Biol* 1997;17:1539-44.
  42. Smith FB, Lee AJ, Fowkes FGR, Price JF, Rumley A, Lowe GD. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. *Arterioscler Thromb Vasc Biol* 1997;17:3321-5.
  43. Ariens RA, Philippou H, Nagaswami C, Weisel JW, Lane DA, Grant PJ. The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. *Blood* 2000;96:988-95.
  44. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost* 1998;79:8-13.
  45. Catto AJ, Kohler HP, Coore J, Mansfield MW, Stickland MH, Grant PJ. Association of a common polymorphism in the factor XIII gene with venous thrombosis. *Blood* 1999;93:906-8.
  46. Balogh I, Szoke G, Karpati L, Wartiovaara U, Katona E, Komaromi I, et al. Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. *Blood* 2000;96:2479-86.
  47. Margaglione M, Bossone A, Brancaccio V, Ciampa A, Di Minno G. Factor XIII Val34Leu polymorphism and risk of deep vein thrombosis. *Thromb Haemost* 2000;84:1118-9.
  48. Greaves M, Baglin T. Laboratory testing for heritable thrombophilia: impact on clinical management of thrombotic disease. *Br J Haematol* 2000;109:699-703.
  49. Mannucci PM. Genetic hypercoagulability: prevention suggests testing family members. *Blood* 2001;98:21-2.
  50. Lensen R, Bertina RM, Vandenbroucke JP, Rosendaal FR. High factor VIII levels contribute to the thrombotic risk in families with factor V Leiden. *Br J Haematol* 2001;114:380-6.
  51. Bloemenkamp KW, Helmerhorst FM, Rosendaal FR, Vandenbroucke JP. Venous thrombosis, oral contraceptives and high factor VIII levels. *Thromb Haemost* 1999;82:1024-7.
  52. Denson KWE, Biggs R. Laboratory diagnosis, tests of clotting function and their standardization. In: R Biggs, Editor. *Human Blood Coagulation Haemostasis and Thrombosis*. Oxford, Blackwell Scientific Publication; 1972. p. 278-332.
  53. Hemker HC, Beguin S. Phenotyping the clotting system. *Thromb Haemost* 2000;84:747-51.
  54. Kyrle PA, Mannhalter C, Beguin S, Stumpflen A, Hirschl M, Weltermann A, et al. Clinical studies and thrombin generation in patients homozygous or heterozygous for the G20210A mutation in the prothrombin gene. *Arterioscler Thromb Vasc Biol* 1998;18:1287-91.
  55. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C. Prediction of a cofactor for activated protein C. *Proc Natl Acad Sci USA* 1993;90:1004-8.
  56. de Visser MC, Rosendaal FR, Bertina RM. A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. *Blood* 1999;93:1271-6.
  57. Marcucci R, Abbate R, Fedi S, Gori AM, Brunelli T, Bruni V, et al. Acquired activated protein C resistance in postmenopausal women is dependent on factor VIII:c levels. *Am J Clin Pathol* 1999;111:769-72.
  58. Tripodi A, Chantarangkul V, Mannucci PM. Hyperprothrombinemia may result in acquired activated protein C resistance. *Blood* 2000;96:3295-6.
  59. Rodeghiero F, Tosetto A. Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. *Ann Intern Med* 1999;130:643-50.
  60. Rosing J, Tans G, Nicolaes GA, Thomassen M, van Oerle R, van der Ploeg P, et al. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. *Br J Haematol* 1997;97:233-8.
  61. Rosing J, Middeldorp S, Curvers J, Christella M, Thomassen LG, Nicolaes GA, et al. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomized cross-over study. *Lancet* 1999;354:2036-40.

#### *Manuscript processing*

This manuscript was peer-reviewed by two external reviewers and by Professor Mario Cazzola, who accepted this paper for publication. Manuscript received on March 28, 2003; accepted on April 14, 2003.