Review

Synthèse

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Criteria for evaluating evidence that laboratory abnormalities are associated with the development of venous thromboembolism

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Abstract

THE IDENTIFICATION OF CONDITIONS ASSOCIATED WITH an increased risk of venous thromboembolism may indicate the need for aggressive prophylaxis during periods of high risk, prolonged anticoagulant therapy after an initial venous thromboembolic episode, the investigation of asymptomatic family members and the avoidance of oral contraceptives. Advances in laboratory medicine have led to the identification and assessment of many proteins responsible for normal hemostasis, and associations between abnormalities in a number of these proteins and venous thromboembolism have been reported. Without the ability to appraise this information critically, physicians may be unable to determine whether or how they should modify their clinical practice. Criteria for determining whether specific laboratory abnormalities have a relationship with venous thromboembolism are proposed here, and one example of the application of these guidelines is provided.

Venous thromboembolism (VTE) can be subclassified as being associated with either a short-term (e.g., post hip surgery) or long-term (e.g., metastatic cancer) clinical risk factor, a laboratory abnormality (e.g., antithrombin [AT] deficiency), or there may be no associated factor (idiopathic).

The development of "screening for thrombophilia" (an increased tendency to develop VTE) was driven by the observation that some young individuals with recurrent VTE and no other associated risk factors had a strong family history of VTE, suggesting an autosomal dominant genetic defect.[1] Subsequently, AT was discovered, and an inherited deficiency was characterized that causes AT levels to be about one-half of the norm and leads to a predisposition to recurrent VTE.[2–6] Other proteins responsible for normal hemostasis have since been identified. Assays that measure peptides or enzyme-inhibitor complexes reflecting thrombin generation or activity have become routinely available, as have "global tests" that reflect interactive cascades. Associations between abnormalities in the results of many of these tests and VTE have been reported. Laboratory parameters that are considered to be useful in diagnosing thrombophilia are summarized in Table 1.

Many centres now routinely test young patients for thrombophilia after one episode of thrombosis or when there is a family history of thrombosis. Well-designed prospective studies show that the risk of recurrent VTE differs in the subgroups of patients described earlier.[7–11] Patients with a short-term risk factor have a low risk of recurrence after 3 months of anticoagulants.[8–11] Patients who develop idiopathic VTE and those with a persistent risk factor have a higher risk of recurrence,[7–11] necessitating prolonged anticoagulation. Many experts recommend long-term anticoagulants when VTE is associated with a laboratory abnormality,[12] although this approach has not been evaluated in clinical trials. Testing is also performed to identify individuals who might benefit from more aggressive prophylaxis in situations associated with an increased risk of VTE, for example, pregnant women with AT deficiency.[12–14]

The role of many of these laboratory abnormalities in predicting VTE is unclear, and an abnormal assay with an unproven link to VTE might mislead clinicians into

inappropriate decision-making. In this review we propose criteria for determining whether laboratory abnormalities have a relationship with the development of VTE, and we apply the criteria to a recently discovered laboratory assay.

Levels of evidence for assessing the significance of laboratory abnormalities in VTE

Many studies suggest that the inappropriate ordering of tests is a significant problem in several clinical settings.[15–20] When evaluating the usefulness of a laboratory assay in diagnosing thrombophilia, the credibility of the assay, the strength of the association between VTE and the laboratory abnormality, and the evidence for a causal relationship between the 2 must be considered. Elements relevant to assessing causation[20] and information contained in previously published "User's Guides to the Medical Literature" that evaluate harm associated with a particular exposure[21] can be used in this situation. The following criteria may prove helpful for evaluating laboratory assays reported to diagnose thrombophilia.

Table 1: Laboratory parameters that may be useful in the diagnosis of thrombophilia

Variables of thrombin and fibrin(ogen) turnover
Prothrombin fragment 1.2
D-dimer
Thrombin-antithrombin (TAT) complexes
Soluble fibrin
Activated protein C (APC) sensitivity ratio (endogenous thrombin potential)
Procoagulant variables
Fibrinogen
Factor V Leiden
Factor VII
Factor VIII (antigen/activity)
Von Willebrand factor activity
Factor XI
Prothrombin variant 20210
Anticoagulant variables
Antithrombin (antigen/activity)
Protein C (antigen/activity)
Protein S (total/free)
Protein S (activity)
Activated protein C (APC) sensitivity
Profibrinolytic variables
Plasminogen
Tissue plasminogen activator (t-PA) (antigen/activity)
Plasmin-antiplasmin complex
Global fibrinolysis tests
Antifibrinolytic variables
Plasminogen activator inhibitor (PAI-1) (antigen/activity)
Miscellaneous
Cortisol-binding globulin
C-reactive protein
Homocysteine
Antiphospholipid antibody

1. Is the laboratory assay measured in a credible fashion?

To assess an assay's credibility, 4 questions must be answered.

a. *Has the assay been performed appropriately?* The assay should be performed using standardized, reproducible methodology and careful quality control with an appropriate spectrum of patients and controls. A broad range of people with disease is required to assess the assay's sensitivity adequately, whereas a broad spectrum of people without disease is necessary to assess its specificity.[22] Reagents, laboratory procedures, analytic methods and study populations should be clearly described, so that others can repeat the assay.
b. *Has the accuracy of the analytic method been described?* Inaccurate measurements may obscure true relationships; therefore, the extent to which the assay measures what it is intended to measure should be made clear, usually by comparison with a reference standard.
c. *Has the variability of the assay been described?* Biologic variation leads to differences among individuals of different age, sex, place of origin or disease status. Variation can also occur within an individual over time. When measurements are repeated, variation can result from random error.
d. *Have the results been verified?* Because technical problems such as improper venipuncture technique, sample processing or assay procedure can affect results, tests with abnormal results should always be repeated.

2. Is there supporting evidence from clinical or observational investigations that the abnormality is associated with VTE?

Since randomized trials are not available in this area, cross-sectional family studies, cohort studies or case–control studies, or all 3, constitute the usual evidence. Relevant criteria are listed below.

a. Was the comparison group similar regarding important determinants of VTE other than the one of interest?
b. Were exposures and outcomes measured similarly in the groups?
c. Was follow-up sufficiently long and complete?
d. How strong is the association between exposure and outcome?
e. How precise is the estimate of the risk?

3. Have potential confounders been ruled out as causes for the observation?

Even if the laboratory measurements are valid, a com-

parison between groups for a given attribute can be biased if some extraneous factor that can affect that attribute independently is unequally distributed. Systematically biased measurements may either create or obscure associations.

Confounders may alter laboratory results. Normal values of components of the hemostatic system vary with age,[23] sex and nutritional status. Heparin administration[24] or disseminated intravascular coagulation,[25] or both, have been associated with temporary reductions in AT levels. Similarly, coumarin derivatives cause low levels of proteins C and S.[26–28] Liver disease affects many hemostatic factors. Therefore, when a laboratory abnormality occurs, the test should be repeated once the confounder(s) has (have) resolved.

Other confounders may independently alter the risk of developing VTE. Clinical studies of VTE should control for concomitant laboratory abnormalities independently associated with VTE as well as clinical confounders, such as recent trauma or surgery.

4. Is the magnitude of the abnormality sufficient to explain thrombophilia?

A minimum clinically important alteration in levels of laboratory variables appears necessary to increase the risk of thrombosis in individuals with congenital thrombophilia. For example, symptomatic individuals with a congenital AT deficiency have levels that are approximately 50% of the norm. For most acquired abnormalities, the critical level at which the risk of VTE is increased is unknown. A statistically significant decrease in AT levels of 10% (from 100% to 90%) associated with oral contraceptive use[29] has been advanced as an explanation for its association with VTE. The clinical relevance of such an alteration is dubious in view of the 50% reduction noted in symptomatic individuals with inherited AT deficiency, and because the AT levels are still well within the normal range (about 80% and above). On the other hand, patients with severe liver disease may have AT levels of 50%, however, because of concomitant reductions in procoagulants, they usually bleed rather than develop VTE (see earlier section on confounders).

5. Does the link between the laboratory abnormality and VTE make biologic sense?

The association should be plausible and consistent with current knowledge.

a. *Does it fit with a known pathogenic mechanism?* Although for some abnormalities biologic plausibility is obvious (e.g., AT deficiency), for others it is unclear (e.g., antiphospholipid antibodies). There is a clear and strong association between antiphospholipid antibodies and VTE,[30,31] and several "cause-and-effect" mechanisms have been suggested,[30] however, none has emerged as the "final answer." In this situation, if a clinical association is convincing, it is important to determine whether the abnormality may cause VTE, through an as yet undetermined mechanism, or whether the abnormality is merely associated with VTE.
b. *Is the temporal relationship correct?* The laboratory abnormality should precede the episode and be predictive of VTE, rather than be a consequence of VTE.
c. *Is there a biologic gradient?* Demonstrating a graded effect on outcome with different degrees of exposure often increases the probability of causality. This type of "dose–response" relationship has been demonstrated in several inherited thrombophilias. For example, the thrombotic risk for individuals with Factor V Leiden appears to be greater for homozygotes than for heterozygotes.[32,33] However, steady increases in relative risk (a constant slope) are not necessary to demonstrate a biologic gradient, because threshold, ceiling, optimum and nonlinear graded effects are also possible. For example, in patients with hyperhomocysteinemia, the risk of thrombosis appears substantially increased at the highest plasma homocysteine levels, indicating a threshold effect.[34]

Summary

The strength of the evidence available to support an assay's role in diagnosing thrombophilia should be described. The more criteria an assay meets, the more likely its validity. However, not all criteria are equally important. Biologic plausibility is less important than credible laboratory technique. Therefore, it is not sufficient to simply tally the number of criteria met in order to describe the strength of the evidence available to support an assay's diagnostic role. Fig. 1 shows an algorithm for assessing levels of evidence. At a minimum, we suggest that there should be supporting evidence that the abnormality is measured using credible laboratory techniques and is associated with VTE (level III evidence). If the magnitude of the abnormality is sufficient to explain thrombophilia and the elimination of potential laboratory and clinical confounders has been achieved, this strengthens the available evidence (level II evidence). Level I evidence implies that all the criteria for level II have been met *and* there is biologic plausibility for an association.

Application of criteria

Induced resistance to the activated protein C (APC) anticoagulant system, as diagnosed by the APC sensitivity ratio (APC-sr) assay,[35] has been proposed by some to explain the stronger association between VTE and the third-generation oral contraceptives than between VTE and the second-generation oral contraceptives demonstrated in some,[36–38] but not all,[39–41] studies. For illustrative purposes, the validity of this conclusion will undergo an initial assessment by determining whether this assay meets the criteria for association with VTE.

1. Is the laboratory assay measured in a credible fashion?

The APC-sr has been evaluated in young healthy volunteers, users and nonusers of oral contraceptives, individuals with a history of VTE and carriers of the Factor V Leiden mutation. Pregnant women and patients with intercurrent diseases have not been assessed. Other than in those initiating and discontinuing oral contraception, the variability of the APC-sr in a given individual over time has not been described. Although the assay has been independently and consistently reproduced in another laboratory,[42] others have had difficulty replicating the methods (Marilyn Johnston, Hamilton Civic Hospitals Research Centre, Hamilton, Ont.; personal communication, 1999).

2. Is there supporting evidence from clinical or observational investigations that the abnormality is associated with VTE?

The available evidence is conflicting. In a case–control study of 172 consecutive men with a first, objectively confirmed episode of deep vein thrombosis and 201 age- and sex-matched controls,[43] the APC-sr predicted risk of venous thrombosis in the highest quartile of the ratio compared with the lowest quartile of the ratio, even after exclusion of Factor V Leiden carriers, (odds ratio [OR] 3.4, 95% confidence interval [CI] 1.1–10.8; $p < 0.05$).[43] However, another case–control study involving 67 women with confirmed VTE and 290 age-matched controls found no association between VTE and APC-sr (OR 0.65, 95% CI 0.35–1.22).[42] Exclusion of Factor V Leiden–positive women and oral contraceptive users did not change the results significantly.

3. Have potential confounders been ruled out as causes for the observation?

Although the APC-sr predicted risk of thrombosis after exclusion of Factor V Leiden carriers in the first study described above,[43] other abnormalities associated with abnormal APC-sr results (protein C or S deficiency) were not excluded.

4. Is the magnitude of the abnormality sufficient to explain thrombophilia?

In women taking the third-generation oral contraceptive, the APC-sr results were similar to those seen in Factor V Leiden heterozygotes and significantly higher than those in subjects taking second-generation oral contraceptives;[35] therefore, the magnitude of the abnormality could be sufficient to explain thrombophilia. However, as noted earlier, since this is an acquired abnormality, one cannot conclude that similar decrements to those observed in a congenital abnormality confer a similar magnitude of (or any) thrombophilia.

5. Does the link between the laboratory abnormality and VTE make biologic sense?

a. Does it fit with a known pathogenic mechanism? The assay measures abnormalities in the protein C pathway. Other abnormalities of this pathway such as protein C and protein S deficiency, as well as activated protein C resistance because of Factor V Leiden, have been associated with VTE. Therefore, a link between an abnormal APC-sr and VTE is biologically plausible.

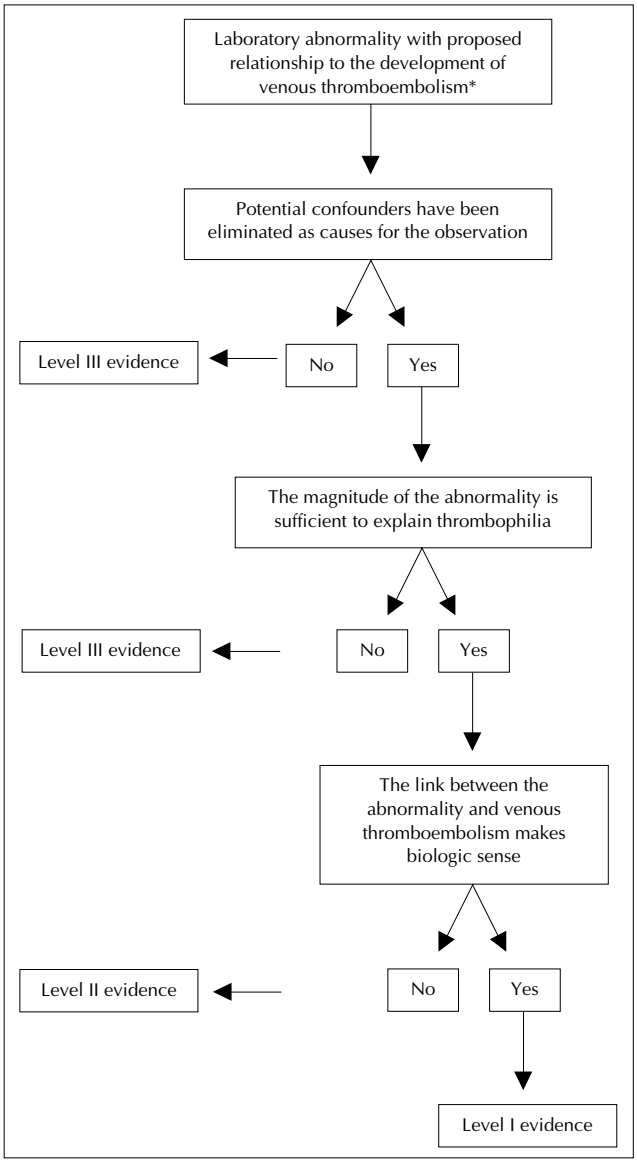

Fig. 1: Levels of evidence for evaluating the relationship between laboratory abnormalities and venous thrombophilia. *The laboratory abnormality has been measured in a credible fashion, and there is supporting evidence for an association between the abnormality and venous thromboembolism from clinical or observational studies.

b. Is the temporal relationship correct? The results of APC-sr have been shown to increase with initiation of oral contraceptive therapy in women without VTE.[44]

c. Is there a biologic gradient? Appropriate studies have not been performed in order to determine whether the risk of VTE is related to the APC-sr.

The association between APC-sr and thrombophilia remains controversial. On the one hand, there is biologic plausibility, and the test appears to be capable of distinguishing subjects who have Factor V Leiden from those who do not. On the other hand, a number of criteria have not been met by the APC-sr. Although the assay has been independently reproduced by one group, others have had difficulty with the assay (Marilyn Johnston, Hamilton Civic Hospitals Research Centre, Hamilton, Ont.; personal communication, 1999). Furthermore, given the conflicting clinical evidence regarding an association between the abnormality and VTE, there is insufficient evidence, using our algorithm, to support the diagnostic role of this assay. Until these issues have been addressed, we believe it is premature to conclude that this assay is useful in diagnosing thrombophilia.

Conclusion

The clinician will continue to be inundated with new laboratory tests that are said to predict VTE. Without critically appraising the available information, physicians may be unable to determine whether or how they should modify their clinical practice. We have proposed criteria to assist physicians in using information from studies to estimate the usefulness of laboratory tests in the diagnosis of venous thrombophilia.

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References

1. Jordan FLJ, Nandorff F. The familial tendency in thromboembolic disease. *Acta Med Scand* 1956;156:267-75.
2. Egeberg O. Inherited antithrombin deficiency causing thrombophilia. *Thromb Diath Haemorrh* 1965;13:576-300.
3. Demers C, Ginsberg JS, Hirsh J, Henderson P, Blajchman MA. Thrombosis in antithrombin III deficient persons: report of a large kindred and literature review. *Ann Intern Med* 1992;116:754-61.
4. Olds RJ, Lane DA, Chowdhury V, Defano V, Leone G, Thein SL. Complete nucleotide sequence of the antithrombin gene: evidence for homologous recombination causing thrombophilia. *Biochem* 1993;32:4216-24.
5. Lane DA, Kunz G, Old RJ, Thein SL. Molecular genetics of antithrombin deficiency. *Blood Rev* 1996;10:59-74.
6. Lane DA, Olds RJ, Boisclair M, Chowdhury V, Thein SL, Cooper DN, et al. Antithrombin III mutation database: first update. *Thromb Haemost* 1993;70: 361-9.
7. Kearon C, Gent M, Hirsh J, Weitz J, Kovacs JM, Anderson DR, et al. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. *N Engl J Med* 1999;340:901-7.
8. Research Committee of the British Thoracic Society. Optimum duration of anticoagulation for deep-vein thrombosis and pulmonary embolism. *Lancet* 1992;340:873-6.
9. Schulman S, Rhedin AS, Lindmarker P, Carlsson A, Lators G, Nicol P, et al, and the Duration of Anticoagulation Trial Study Group. A comparison of six weeks with six months of oral anticoagulant therapy after a first episode of venous thromboembolism. *N Engl J Med* 1995;332:1661-5.
10. Prandoni P, Lensing AWA, Cogo A, Cuppini S, Villalta S, Carta M, et al. The long-term clinical course of acute venous thrombosis. *Ann Intern Med* 1996;125:1-7.
11. Levine MN, Hirsh J, Gent M, Turpie AG, Weitz J, Ginsberg J, et al. Optimal duration of oral anticoagulant therapy: a randomized trial comparing four weeks with three months of warfarin in patients with proximal deep vein thrombosis. *Thromb Haemost* 1995;74:606-11.
12. Mannucci PM, Tripodi A. Laboratory screening of inherited thrombotic syndromes. *Thromb Haemost* 1987;51:247-51.
13. Hirsh J, Prins MN, Samama M. Approach to the thrombophilic patient. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, editors. *Hemostasis and thrombosis: basic principles and clinical practice.* 3rd ed. Philadelphia: JB Lippincott; 1994. p.1543-61.
14. Van den Belt AGM, Prins MN, Huismann MN, Hirsh J. Familial thrombophilia: a review analysis. *Clin Appl Thrombosis/Hemostasis* 1996;2:227-36.
15. Bareford D, Hayling A. Inappropriate use of laboratory services: long-term combined approach to modify request patterns. *BMJ* 1990;39:1305-7.
16. Dans PE, Cafferty L, Otter SE, Johnson RJ. Inappropriate use of cerebrospinal fluid Venereal Disease Research Laboratory (VDRL) test to exclude neurosyphilis. *Ann Intern Med* 1986;104:86-9.
17. Siegel DL, Edelstein PH, Nachamkin I. Inappropriate testing for diarrheal diseases in the hospital. *JAMA* 1990;263:979-82.
18. Hyams KC. Inappropriate urine cultures in hospitalized patients receiving antibiotic therapy. *Arch Intern Med* 1987;147:48-9.
19. Bush TM, Shlotzhauer TL, Grove W. Serum complements: inappropriate use in patients with suspected rheumatic disease. *Arch Intern Med* 1993;153: 2363-6.
20. Hill AB. The environment and disease: association or causation? *Proc R Soc Med* 1965;58:295-300.
21. Levine M, Walter S, Lee H, Haines T, Holbrook A, Moyer V, for the Evidence-Based Medicine Working Group. Users' guide to the medical literature. IV. How to use an article about harm. *JAMA* 1994;271:1615-19.
22. Ransohoff DF, Feinstein AR. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. *N Engl J Med* 1978;199:926-30.
23. Andrew M. Developmental hemostasis: relevance to thromboembolic complications in pediatric patients. *Thromb Haemost* 1995;74:415-25.
24. Marciniak E, Gockerman JP. Heparin-induced decrease in circulating antithrombin III. *Lancet* 1997;ii:581-4.
25. Schipper HG, Jenkins CS, Kahle LH, ten Cate JW. Antithrombin-III transfusion in disseminated intravascular coagulation. *Lancet* 1978;ii:854.
26. Mannucci PM, Viagano S. Deficiencies of protein C, an inhibitor of blood coagulation. *Lancet* 1982;2:463-6.
27. Vigano-D'Angelo S, Comp PC, Esmon CT, D'Angelo A. Relationship between protein C antigen and anticoagulant activity during oral anticoagulation and in selected disease states. *J Clin Invest* 1986;77:416-25.
28. Takahashi H, Wada K, Hayashi S. Behavior of protein S during long-term oral anticoagulant therapy. *Thromb Res* 1988;51:241.
29. Weekink GH, Kahle LH, Lamping RJ, ten Cate JS, Treffers PE. Antithrombin III in oral contraceptive users and during normotensive pregnancy. *Acta Obstet Gynecol Scand* 11984;63:57-61.
30. Long AA, Ginsberg JS, Brill-Edwards P, Johnston M, Turner C, Denburg JA, et al. The relationship of antiphospholipid antibodies to thromboembolic disease in systemic lupus erythematosus: a cross-sectional study. *Thromb Haemost* 1991;66:520-4.
31. Feinstein DI. Immune coagulation disorders. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, editors. *Hemostasis and thrombosis: basic principles and clinical practice.* 3rd ed. Philadelphia: JB Lippincott; 1994. p. 881-905.
32. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 1995;85:1504-8.

33. Zoller B, Svensson PJ, He X, Dahlback B. Identification of the same factor V mutation in 47 out of 60 thrombosis-prone families with inherited resistance to activated protein C. *J Clin Invest* 1994;94:2521-4.
34. den Heijer M, Koster T, Blom HJ, Bos GMJ, Briet E, Reitsma PH, et al. Hyperhomocysteinemia as a risk factor for deep vein thrombosis. *N Engl J Med* 1996;34:759-62.
35. Rosing J, Tans G, Nicolaes GAF, Thomassen MCL, van Oerle R, ven der Ploeg PMEN, 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.
36. Spitzer WO, Lewis MA, Leinemann LA, Thorogood M, MacRae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case control study (Transnational Research Group on Oral Contraceptives and the Health of Young Women). *BMJ* 1996;321:83-8.
37. Jick H, Jick SS. Gurewich V, Myers MW, Vasiliakis C. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing prostagen components. *Lancet* 1995;346:1589-93.
38. World Health Organization. Effect of different prostagens in low oestrogen oral contraceptives on venous thromboembolic disease: World Health Organization collaborative study of cardiovascular disease and steroid hormone contraception. *Lancet* 1995;346:1582-8.
39. Farmer RDT, Lawrenson RA, Thompson CR, Kennedy JG, Hambleton IR. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. *Lancet* 1997;349:83-8.
40. Lewis MA, MacRae KD, Kuhl-Habichl D, Bruppacher R, Heinemann LA, Spitzer WO. The differential risk of oral contraceptives: the impact of full exposure history. *Hum Reprod* 1999;14:1493-6.
41. Suissa S, Blais L, Spitzer WO, Cusson J, Lewis M, Heinemann L. First-time use of newer oral contraceptives and the risk of venous thromboembolism. *Contraception* 1997;56:141-6.
42. Heinemann LA, Assmann A, Spannagle M, Schramm W, Dick A, Kluft C, et al. Normalized activated protein C Ratio itself not associated with increased risk of venous thromboembolism. *Contraception* 1998;58:321-2.
43. Tans G, Rosendaal FR, Curvers J, Thomassen MCL, Bertina RM, Rosing J. APC resistance determined with the endogenous thrombin generation potential is associated with venous thrombosis: a blinded clinical evaluation [abstract 640]. XVIIth Congress of the International Society on Thrombosis and Haemostasis; 1999 Aug 14-21; Washington. *Thromb Haemost* 1999;Aug (Suppl):202.
44. Rosing J, Middeldorp S, Corvers J, Thomassen MCL, Nicolaes GAF, Meijers JCM, et al. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. *Lancet* 1999;354:2036–40.

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