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Reduction of high fetal loss rate by anticoagulant treatment during pregnancy in antithrombin, protein C or protein S deficient women

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Abstract

Hereditary thrombophilia is associated with an increased risk of fetal loss. Assuming that fetal loss is due to placental thrombosis, anticoagulant treatment might improve pregnancy outcome. In an observational family cohort study, we prospectively assessed the effects of anticoagulant drugs on fetal loss rates in women with hereditary deficiencies of antithrombin, protein C or protein S. The cohort contained 376 women (50 probands and 326 deficient or non-deficient relatives). Probands were consecutive deficient patients with venous thromboembolism. Thromboprophylaxis during pregnancy was recommended in deficient women, irrespective of prior venous thromboembolism, and in non-deficient women with prior venous thromboembolism. Outcome of first pregnancy was analysed in 55 eligible women. Of 37 deficient women, 26 (70%) received thromboprophylaxis during pregnancy, compared with three of 18 (17%) non-deficient women. Fetal loss rates were 0% in deficient women with thromboprophylaxis versus 45% in deficient women without ($P = 0.001$) and 7% in non-deficient women without thromboprophylaxis ($P = 0.37$). The adjusted relative risk of fetal loss in women who received thromboprophylaxis versus women who did not was 0.07 (95% confidence interval 0.001 – 0.7; $P = 0.02$). Our data suggest that anticoagulant treatment during pregnancy reduces the high fetal loss rate in women with hereditary deficiencies of antithrombin, protein C or protein S.

Introduction

Fetal loss is a major problem in human pregnancy. Thrombosis of the uteroplacental vessels is one of the pathophysiological mechanisms that are presumed to cause fetal loss.¹ Changes in blood coagulation and fibrinolysis during normal pregnancy induce a state of hypercoagulability, which predisposes to the development of thrombosis.² Hereditary thrombophilic defects are associated with an increased risk of venous thromboembolism.³⁻⁵ In combination with the physiological changes during pregnancy, these defects may increase the risk of uteroplacental thrombosis and hence the risk of fetal loss.⁶ Although the results from previous studies are contradictory, there is evidence of an increased risk of fetal loss associated with various thrombophilic defects, including hereditary deficiencies of antithrombin, protein C and protein S, factor V Leiden and prothrombin G20210A.⁷⁻⁹

Considering uteroplacental thrombosis as a cause of fetal loss, it is rational to use anticoagulant drugs for its prevention. A benefit of these drugs might particularly be expected in women with deficiencies of antithrombin, protein C or protein S, as these thrombophilic defects are strong risk factors for thrombosis.⁵ Of four previously reported studies,¹⁰⁻¹³ three suggested an improved pregnancy outcome in women with various thrombophilic defects, who received anticoagulant treatment during pregnancy.¹⁰⁻¹²

We performed a prospective, observational study to assess the effects of anticoagulant drug treatment during pregnancy on the risk of fetal loss in a cohort of women with hereditary deficiencies of either antithrombin, protein C or protein S.

Materials and Methods

Study population

Subjects were recruited from a previous large family cohort study, which was designed to estimate the risk of venous thromboembolism in relatives with deficiencies of either antithrombin, protein C or protein S, when compared with non-deficient relatives.¹⁴ In that study, probands were consecutive patients with documented proximal deep vein thrombosis or pulmonary embolism in whom one of these deficiencies was demonstrated. They were referred with clinically suspected venous thromboembolism to our thrombosis out-patient clinic over a period of 12 years. First-degree relatives 15 years of age or older were identified by pedigree analysis. As the number of antithrombin deficient probands was small, second degree relatives from a deficient parent were also identified. Probands and relatives were enrolled after informed consent was obtained. Detailed information on episodes of venous thromboembolism, external risk factors for thrombosis, anticoagulant treatment and, in women, their obstetric history, was collected using a standardised questionnaire¹⁵

and by reviewing medical records. Blood samples for testing on thrombophilic defects were taken after clinical data had been collected.

In the present study, female probands and relatives were followed prospectively from the time they were classified as deficient or non-deficient. The data recorded during the follow-up period included episodes of venous thromboembolism, use of anticoagulants, number of pregnancies and pregnancy complications. Women were evaluable if they became pregnant before the end of study. Women with only terminated or ectopic pregnancies were excluded from analysis. Thromboprophylaxis during pregnancy and the 6-week period after delivery was recommended in all women with a deficiency, regardless of prior venous thromboembolism, and in non-deficient women only if they had a history of venous thromboembolism. Thromboprophylaxis was started as soon as a pregnancy test was positive. It consisted of unfractionated (UF) or low-molecular-weight (LMW) heparin before 16 weeks and after 36 weeks of gestation, and a vitamin K antagonist between 16 and 36 weeks and after delivery. Over time, LMW heparin therapy during the entire pregnancy and puerperium became common practice. Doses were used as in the treatment of venous thromboembolism.

In contrast to heparins, vitamin K antagonist cross the placenta and can lead to embryopathy and fetal bleeding. Embryopathy includes nasal hypoplasia, stippled epiphysis and central nervous system abnormalities, such as optic atrophy. To prevent these effects, vitamin K antagonists are replaced by heparins.¹⁶ LMW heparins are preferred to UF heparin because the former are rarely associated with heparin-induced thrombocytopenia and osteoporosis. Both heparins and vitamin K antagonists are not excreted in breast milk and can be safely given to nursing mothers.¹⁶

Fetal loss was defined as early fetal loss if it occurred within 22 weeks of gestation, or as late fetal loss after more than 22 weeks of gestation, according to the criteria of the World Health Organization.¹⁷ This study was approved by the institutional review board of our hospital.

Laboratory studies

Protein C and protein S antigen levels were measured by enzyme-linked immunosorbent assay (ELISA) (reagents obtained from DAKO, Glostrup, Denmark), activity of protein C (Berichrom Protein C; Dade Behring, Marburg, Germany) and antithrombin (Coatest; Chromogenix, Mölndal, Sweden) by chromogenic substrate assays. Normal ranges [mean \pm 2 standard deviations (SD)] were determined in 393 healthy blood donors, who had no (family) history of venous thromboembolism and were neither pregnant, nor had used oral contraceptives for at least 3 months. Blood donors were balanced for age and sex. Antithrombin deficiency was defined by lowered levels of antithrombin activity (<74 IU/dl), protein C deficiency type I and type II by lowered levels of either protein C antigen (<63 IU/dl) and/or activity (<64 IU/dl), and protein S deficiency type I was defined by lowered total (<67 IU/dl) and free protein S levels (<65 IU/dl). Deficiencies were considered inherited if confirmed at re-

peated measurement of samples collected at 3-month intervals and demonstrated in at least two family members, while acquired conditions were excluded. A deficiency was considered acquired and due to oral contraception or pregnancy, unless it was confirmed at least 3 months after discontinuation of oral contraception and delivery respectively. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions.³⁻¹⁸ Factors VIII:C, IX:C and XI:C were measured by one-stage clotting assays (Amelung GmbH, Lemgo, Germany) and were increased at levels above 150 IU/dl.¹⁹ Lupus anticoagulant was demonstrated by abnormal dilute Russell viper venom time, activated partial thromboplastin time and tissue thromboplastin inhibition, that normalised by adding phospholipids.²⁰ Fasting and post-methionine-loading levels of homocysteine were measured by high performance liquid chromatography.²¹ Hyperhomocysteinaemia was defined as a fasting level above 18.5 $\mu\text{mol/l}$ and/or a post-loading level above 58.8 $\mu\text{mol/l}$, as described in the Dutch population.²²

In probands and relatives who had venous thromboembolism, blood samples were collected at least 3 months after the event had occurred. If they were still treated at that time with vitamin K antagonists, samples were taken after this therapy had been interrupted for at least 2 weeks, meanwhile nadroparin was given subcutaneously.

Statistical analysis

The effect of thromboprophylaxis on the fetal loss rate was assessed by comparing the outcome of the first pregnancy after enrolment in deficient and non-deficient women who received thromboprophylaxis versus deficient and non-deficient women who did not.

Random effects logistic regression was used to adjust for clustering of women in families. In addition, covariates (age > 35 years, a history of fetal loss prior to enrolment) were considered in multivariate analysis if univariate analysis showed an association at a P-value < 0.20. Continuous variables were expressed as mean values and SD or median values and ranges, and categorical data as counts and percentages. Differences between groups were evaluated by the Student's t-test or Mann-Whitney U-test for continuous data and by Fisher exact test for categorical data, depending on the normality of data. A two-tailed P-value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed using SAS software, version 9.1 (SAS-Institute Inc., Cary, NC, USA).

Results

Overall, of 50 female probands and 326 female relatives, 286 were eligible i.e. alive and 15 years of age or older (Table 1).

Table 1. Recruitment of study population.

	Antithrombin- deficient families (n=12)	Protein C-deficient families (n=40)	Protein S-deficient families (n=39)	Total (n=91)
Female probands	7	21	22	50
Female relatives	95	120	111	326
All females	102	141	133	376
Eligible females	73	115	98	286
No consent				
Geographic reasons	1	2	0	3
Refusal	2	3	4	9
No pregnancy	53	87	78	218
Only terminated pregnancies	0	1	0	1
Evaluable females	17	22	16	55
Deficient	12	13	12	37
Protein levels, IU/dl; mean (SD)	55 (8)	55 (16)	50 (9)	53 (11)
Non-deficient	5	9	4	18
Protein levels, IU/dl; mean (SD)	95 (8)	100 (18)	84 (7)	96 (15)

SD, standard deviation.

Consent was refused or not obtained due to geographic reasons in 12 relatives (response rate 96%) and 219 women were excluded because they had no pregnancies (218) or only terminated pregnancies (1). The remaining 55 women were evaluable, of whom 37 were deficient and 18 non-deficient. Their characteristics are summarised in Table 2. Fourteen of 37 deficient women and eight of 18 non-deficient women had been pregnant and two women in each group had fetal loss prior to enrolment. Venous thromboembolism had occurred prior to enrolment in 57% of deficient women, compared with 6% of non-deficient women ($P < 0.001$).

Of 37 deficient women, 26 (70%) received thromboprophylaxis during pregnancy (18/21 with previous venous thromboembolism and 8/16 without a history of venous thromboembolism), compared with three of 18 (17%) non-deficient women (one with a history of venous thromboembolism and two without an obvious reason for thromboprophylaxis) (Table 2). Thromboprophylaxis was refused by 11 deficient women; eight had no history of venous thromboembolism and nine had not been pregnant before. Median age (range) at first pregnancy was 23 years (16–31 years) in these women, compared with 28 years (19–33 years) in deficient women who received throm-

boprophylaxis ($P = 0.04$). In non-deficient women, the median age was 26 years (21–30 years) (with thromboprophylaxis) versus 29 years (22–37 years) (without thromboprophylaxis).

Sixty-nine per cent of all women had one or more other thrombophilic defects. These included prothrombin G20210A in 8% of deficient versus 0% of non-deficient women, factor V Leiden in 19% vs. 14%, increased factor VIII levels in 43% versus 62%, increased factor IX levels in 9% vs. 7%, increased factor XI levels in 3% vs. 7% and hyperhomocysteinaemia in 12% vs. 15%. The prevalences of these defects were higher in both deficient and non-deficient women than expected in the normal population, except for high factor IX and XI levels in deficient and non-deficient women, and prothrombin G20210A in non-deficient women (expected: prothrombin G20210A 1–2%; factor V Leiden 5%; increased factor VIII 25%; increased factor IX and factor XI, and hyperhomocysteinaemia, each 10%). None of the differences were statistically significant. Thrombophilic defects were equally distributed among subgroups of women who received thromboprophylaxis or did not (data not shown). Lupus anticoagulant was not found in deficient or non-deficient women.

None of 26 deficient women (0%) with thromboprophylaxis experienced fetal loss, in contrast to five of 11 deficient women (45%) without thromboprophylaxis ($P = 0.001$) (Table 3). Fetal loss rates were comparable in deficient women with thromboprophylaxis (0%) and non-deficient women without thromboprophylaxis (7%) ($P = 0.37$). The relative risk of fetal loss in women who received thromboprophylaxis compared to women without thromboprophylaxis, adjusted for clustering of women in families, was 0.07 [95% confidence interval (CI), 0.01–0.7]. Fetal loss rates when women with and without thromboprophylaxis were compared, were: 0% vs. 63% in antithrombin

Table 2. Characteristics of women with hereditary deficiencies of either antithrombin, protein C or protein S and their non-deficient female relatives.

	Deficient (n = 37)	Non-deficient (n = 18)	P-value
Prior to enrolment			
Pregnant, n (%)	14 (38)	8 (44)	0.8
Fetal loss, n (%)	2 (5)	2 (11)	0.6
Venous thromboembolism, n (%)	21 (57)	1 (6)	<0.001
Follow-up			
Age at enrolment, median (range), years	30 (18–37)	25 (15–47)	0.1
Follow-up period, median (range), years	7.6 (0.5–24)	8.5 (0.07–24)	0.8
Age at first pregnancy, median (range), years	29 (21–37)	27 (16–33)	0.06
Thromboprophylaxis, n (%)	26 (70)	3 (17)	<0.001
LMW heparin, n	10	1	
UF heparin and vitamin K antagonists, n	2	1	
LMW heparin and vitamin K antagonists, n	14	1	

deficient women; 0% vs. 50% in protein C deficient women; and 8% vs. 57% in protein S deficient women. In all cases, fetal loss occurred before 22 weeks of gestation.

Three women experienced venous thromboembolism related to the first pregnancy after enrolment. All were antithrombin deficient and had received thromboprophylaxis. Two events were recurrences of venous thromboembolism. Only one woman, who was protein S deficient, experienced pregnancy-induced hypertension. Complications related to the use of anticoagulants (major bleeds) were not observed.

Table 3. Fetal loss in deficient and non-deficient women, who did or did not receive thromboprophylaxis during pregnancy.

Thromboprophylaxis	Deficient		Non-deficient	
	Yes	No	Yes	No
Pregnancies, n	45	19	4	20
Fetal loss, n (%)	1 (2)	11 (58)	1 (25)	1 (5)
Women, n	26	11	3	15
Fetal loss, n (%)*	0 (0)	5 (45)	1 (33)	1 (7)
	P = 0.001**		P = 0.37**	

*Only first pregnancy analysed.

**Compared with deficient women on thromboprophylaxis.

Discussion

Our study showed that thromboprophylaxis resulted in a 15-fold reduction in fetal loss rate. In deficient women on thromboprophylaxis, fetal loss rate was significantly lower than in deficient women without prophylaxis (0% vs. 45%, $P = 0.001$), while it was comparable with the fetal loss rate in non-deficient women without prophylaxis (0% vs. 7%, $P = 0.37$). The number of non-deficient women on thromboprophylaxis was too small to allow analysis.

Thus far, four studies addressed the effects of anticoagulants on the outcome of pregnancy in women with various thrombophilic defects.¹⁰⁻¹³ In the first study, 50 women with unexplained fetal loss and single or combined thrombophilic defects [protein S deficiency, factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase (MTHFR)] received LMW heparin during their next pregnancy.¹⁰ The fetal loss rate dropped to 25%. In the second study, 33 women with a history of either fetal loss, pre-eclampsia, abruptio placentae or intrauterine growth retardation, and a thrombophilic defect (protein S deficiency, factor V Leiden, prothrombin G20210A or MTHFR), were treated with a prophylactic dose of LMW heparin and low dose aspirin (100 mg/d) during the next pregnancy.¹¹ Recurrent events in the next pregnancy were reported in only 9% of the women. The third study compared a prophylactic dose of LMW heparin to low dose aspirin in 160 women with one prior un-

explained fetal loss after 10 weeks of gestation and a thrombophilic defect (protein S deficiency, factor V Leiden or prothrombin G20210A).¹² Fetal loss rates in this randomised trial, without an untreated or placebo-treated control group, were 14% and 77% in women who were treated with LMW heparin and aspirin respectively. No benefit of anticoagulant treatment was demonstrated in the fourth study.¹³ Twenty-one women with a deficiency of antithrombin, protein C or protein S or factor V Leiden, received heparin or vitamin K antagonists. Indications for this treatment were venous thromboprophylaxis, prevention of fetal loss or unknown. Fetal loss rates were 24% in women with anticoagulant treatment compared with 27% in controls (adjusted relative risk 0.7; 95% CI, 0.2–3.2).

A comparison of these studies with our study is difficult, due to differences in design, selection criteria and thrombophilic defects. The women in our study were not selected for a compromised obstetric history. We recommended treatment with high doses of anticoagulants during pregnancy and puerperium in all deficient women, considering that they were at high risk of venous thromboembolism. This assumption was confirmed by our finding that 57% of deficient women already had experienced venous thromboembolism before they were enrolled at a median age of 30 years, compared with 6% of non-deficient women. Moreover, 5% of deficient women had pregnancy-related venous thromboembolism after enrolment despite thromboprophylaxis. For the same reason, a randomised placebo-controlled clinical trial to assess the effects of anticoagulant drug therapy on fetal loss was, in our opinion, unethical. Nevertheless, one-third of deficient women preferred not to be treated with anticoagulants. As a majority of these women, who were of younger age, had no history of venous thromboembolism, their reluctance to thromboprophylaxis was understandable. However, they showed an excessively high fetal loss rate (45%), suggesting that deficient women are at high risk of both venous thromboembolism and fetal loss. A possible explanation for the high risk is the concomitance of one or more other thrombophilic defects, observed in 69% of deficient and non-deficient women. Single or multiple mild thrombophilic defects, in addition to hereditary deficiencies of antithrombin, protein C and protein S, known as strong thrombotic risk factors, might increase the risk of fetal loss due to placental thrombosis, as we previously demonstrated for the risk of venous thromboembolism.¹⁴

Small numbers of women did not allow reliable estimates of fetal loss rates associated with specific combinations of deficiencies and concomitant thrombophilic defects. As concomitant thrombophilic defects were equally distributed among deficient women who received thromboprophylaxis and deficient women who did not, it is likely that the difference in fetal loss rates would be attributed to thromboprophylaxis.

Other causes of fetal loss, like abnormal karyotype, congenital anomalies and uterine malformation were not investigated. However, these were probably equally distributed among women on thromboprophylaxis and women who did not receive

thromboprophylaxis, while an effect of thromboprophylaxis on these conditions is not plausible.

Small numbers of women and a non-randomised design are the main limitations of this study. Whether a randomised clinical trial on fetal loss is feasible in women who are at high risk of venous thromboembolism, remains a matter of debate. It may be easier to perform such a clinical trial in women with more prevalent, mild thrombophilic defects. However, as the risk of venous thromboembolism, as well fetal loss is lower in these women, the potential benefit of anticoagulant drug therapy, and consequently its clinical impact, may be limited.

In conclusion, our data suggest that strong thrombophilic defects, like hereditary deficiencies of antithrombin, protein C and protein S, and combinations of these deficiencies with other, mild thrombophilic defects, are associated with a high risk of fetal loss. This risk seems to be reduced by anticoagulant drug therapy during pregnancy.

References

- 1 Rai RS, Regan L, Chitolie A, Donald JG, Cohen H. Placental thrombosis and second trimester miscarriage in association with activated protein C resistance. *Br J Obstet Gynaecol* 1996; 103: 842-844.
- 2 Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. *Thromb Haemost*. 1984; 52: 176-182.
- 3 Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velde PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; 369: 64-67.
- 4 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 an increase in venous thrombosis. *Blood* 1996; 88: 3698-3703.
- 5 Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV, Prandoni P, Bura A, Rejto L, Wells P, Manucci PM, Girolami A, Buller HR, Prins HM. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. *Blood* 1999; 94: 3702-3706.
- 6 Kupfermink MJ. Thrombophilia and pregnancy. *Reprod Biol Endocrinol*. 2003; 1: 111.
- 7 Sanson BJ, Friederich PW, Simioni P, Zanardi S, Hilsman MV, Girolami A, ten Cate JW, Prins MH. The risk of abortion and stillbirth in antithrombin-, protein C-, and protein S-deficient women. *Thromb Haemost* 1996; 75: 387-388.
- 8 Meinardi JR, Middeldorp S, de Kam PJ, Koopman MM, van Pampus EC, Hamulyák K, Prins MH, Buller HR, van der Meer J. Increased risk for fetal loss in carriers of the factor V Leiden mutation. *Ann Intern Med* 1999; 130: 736-739.
- 9 Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, Zournatzi V, Makris PE, Bontis J, Kotsis A. Factor V Leiden and prothrombin G20210A mutations, but not methylenetetra-

- hydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod.* 2000; 15: 458-462.
- 10 Brenner B, Hoffman R, Blumenfeld Z, Weiner Z, Younis JS. Gestational outcome in thrombophilic women with recurrent pregnancy loss treated by enoxaparin. *Thromb Haemost* 2000; 83: 93-97.
 - 11 Kupferminc MJ, Fait G, Many A, Lessing JB, Yair D, Bar-Am A, Eldor A. Low-molecular-weight heparin for the prevention of obstetric complications in women with thrombophilias. *Hypertens Pregnancy* 2001; 20, 35-44.
 - 12 Gris JC, Mercier E, Quere I, Lavigne-Lissalde G, Cochery-Nouvellon E, Hoffet M, Ripart-Neveu S, Tailland ML, Dauzat M, Mares P. Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder. *Blood* 2004; 103: 3695-3699.
 - 13 Vossen CY, Preston FE, Conard J, Fontcuberta J, Makris M, van der Meer FJ, Pabinger I, Palareti G, Scharrer I, Souto JC, Svensson P, Walker ID, Rosendaal FR. Hereditary thrombophilia and fetal loss: a prospective follow-up study. *J Thromb Haemost.* 2004 ; 2: 592-596.
 - 14 Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. *Ann Intern Med.* 2006; 145: 807-815.
 - 15 Frezzato M, Tosetto A, Rodeghiero F. Validated questionnaire for the identification of previous personal or familial venous thromboembolism. *Am J Epidemiol.* 1996; 143: 1257-1265.
 - 16 Bates SM, Greer IA, Hirsh J, Ginsberg JS. Use of antithrombotic agents during pregnancy: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest.* 2004; 126: 627S-644S.
 - 17 Stirrat G.M. Recurrent miscarriage. *Lancet* 1990; 336: 673-675.
 - 18 Danneberg J, Abbes AP, Bruggeman BJ, Engel H, Gerrits J, Martens A. Reliable genotyping of the G-20210-A mutation of coagulation factor II (prothrombin). *Clin Chem* 1998; 44: 349-351.
 - 19 Bank I, Libourel EJ, Middeldorp S, Hamulyák K, van Pampus EC, Koopman MM, Prins MH, van der Meer J, Büller HR. Elevated levels of FVIII:C within families are associated with an increased risk for venous and arterial thrombosis. *J Thromb Haemost.* 2005; 3: 79-84.
 - 20 Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, de Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295-306.
 - 21 Den Heijer M, Blom HJ, Gerrits WB, Rosendaal FR, Haak HL, Wijermans PW, Bos GM. Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis? *Lancet.* 1995; 345: 882-885.
 - 22 Den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med.* 1996; 334: 759-762.

