CLINICAL VALIDITY

Question 18: How often is the test positive when the disorder is present (i.e. sensitivity)?
Question 19: How often is the test negative when the disorder is not present (i.e. specificity)?
Question 20: Are there methods to resolve clinical false positive results in a timely manner?
Question 21: What is the prevalence of the disorder in this setting?
Question 22: Has the test been adequately validated on all populations to which it may be offered?
Question 23: What are the positive and negative predictive values?
Question 24: What are the genotype/phenotype relationships?
Question 25: What are the genetic, environmental or other modifiers?

CLINICAL VALIDITY
Question 18: How often is the test positive when the disorder is present (i.e. sensitivity)?
Question 19: How often is the test negative when the disorder is not present (i.e. specificity)?

Summary

Disorder/Setting

- The specific clinical disorder is recurrent venous thrombosis in individuals with an inherited clotting disorder, and the setting for offering the DNA testing is a confirmed recent episode of deep venous thrombosis in adults (Questions 1 and 3).

Factor V Leiden

- Five studies satisfy the criteria of the present analysis for determining clinical sensitivity and specificity.
- Clinical sensitivity of factor V Leiden testing answers the following question: For every 100 individuals with a recurrent episode of deep venous thrombosis, how many will carry a factor V Leiden mutation?
- The overall clinical sensitivity is 28 percent, with a 95 percent CI of 12.9-34.6%.
- Clinical false positive rate of factor V Leiden testing answers the following question: For every 100 individuals who do not experience a recurrent episode of deep venous thrombosis, how many will carry a factor V Leiden mutation?
- The overall clinical false positive rate (1-specificity) is 19 percent, with a 95 percent CI of 14.1-26.7%.
- The overall likelihood ratio for a recurrent episode of venous thrombosis among factor V Leiden carriers is 1.5.

Prothrombin G20210A

- Four studies satisfy the criteria of the present analysis for determining clinical sensitivity and specificity.
- Clinical sensitivity of prothrombin G20210A testing answers the following question: For every 100 individuals with a recurrent episode of deep venous thrombosis, how many will carry a prothrombin G20210A mutation?
- The overall clinical sensitivity is 11 percent, with a 95 percent CI of 6.2-21.1%.

- Clinical false positive rate of prothrombin G20210A testing answers the following question: For every 100 individuals who do not experience a recurrent episode of deep venous thrombosis, how many will carry a prothrombin G20210A mutation?
- The overall clinical false positive rate (1-specificity) is 6 percent, with a 95 percent CI of 5.6-6.8%.
- The overall likelihood ratio for a recurrent episode of venous thrombosis among prothrombin G20210A carriers is 1.8.

Introduction

In order to answer Question 18 in this review, it is necessary to document factor V Leiden or prothrombin G20210A mutation status in a cohort of individuals with an initial episode of venous thrombosis and then follow the cohort for a period of time to determine who develops a recurrence. Selection criteria for including published studies in the present analysis require a mean of at least four years' follow-up. Five studies satisfy these criteria for factor V Leiden, along with four studies for prothrombin G20210A. All of these studies include only Caucasians (both Hispanic and non-Hispanic). Rates are likely to be different for blacks (Question 22), as the prevalence of factor V Leiden is close to zero.

Sensitivity and Specificity of Factor V Leiden Testing

Question 18 asks how often a positive test for factor V Leiden (FVL) is associated with a recurrent episode of venous thrombosis among individuals who have been diagnosed with an initial episode. This defines the clinical sensitivity of a DNA test (see Appendix for description of 2 x 2 tables and the calculation of sensitivity and specificity). Figure 18-1 shows the mean clinical sensitivity and 95 percent confidence interval among the five selected studies. The confidence interval around the group estimate is calculated using the DerSimonian and Laird random effects model with an accompanying test of heterogeneity. According to the chi-square analysis, these studies are not statistically heterogeneous in their estimate of sensitivity. A total of 1637 individuals was followed for a minimum of 4 years, 385 of whom had a factor V Leiden mutation. The overall sensitivity of factor V Leiden mutation testing for predicting a recurrent episode of venous thrombosis in these studies is 28 percent. Table 18-1 shows the sizes of the individual studies, their respective sensitivities and the 95 percent confidence intervals. Details of the studies used for these calculations are in the Appendix. The Appendix also lists studies excluded from the present analyses because study subjects were not fully confirmed to have a first thrombotic event.

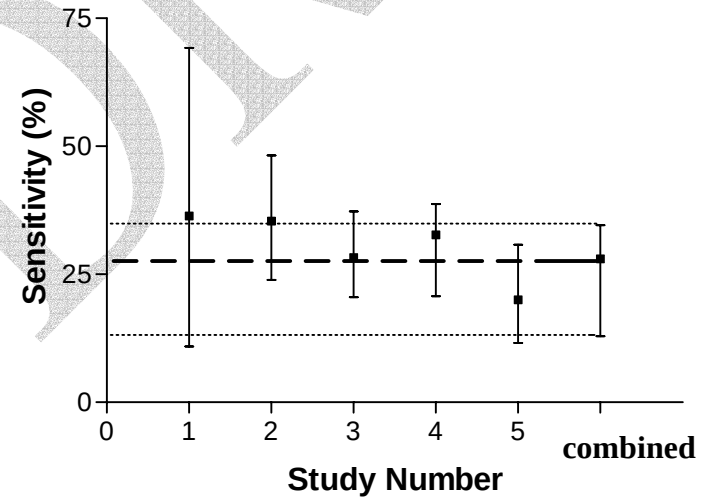

Figure 18-1. Clinical Sensitivity of the Factor V Leiden Mutation Testing. Study reference numbers are from Table 18-1. The individual and overall mean sensitivities are

shown as filled-in squares. Error bars are the 95 percent CI. The combined mean sensitivity is 28 percent (95 percent CI 13-35%). The chi-square for the test for heterogeneity is 3.43 and $p > 0.05$.

Question 19 asks how often a positive test for factor V Leiden is not present among individuals who do not experience a recurrence of venous thrombosis. This defines the clinical specificity of each DNA test. This aspect of clinical test performance can also be expressed as the false positive rate, which is 1-specificity. The false positive rate contributes to understanding test performance by directly expressing how often a mutation will be found among individuals who will not experience a recurrence of venous thrombosis. The false positive rates are shown graphically in Figure 18-2 for the five factor V Leiden studies, and the data on which they are based are shown in Table 18-1. For the five factor V Leiden studies, the overall false positive rate, adjusted for heterogeneity, is 19 percent. The chi-square for the test of heterogeneity in these studies' estimates of the false positive rate is significant, as shown (see Appendix for further discussion of heterogeneity). In addition, the sensitivity divided by the false positive rate gives the likelihood ratio. This is a useful estimate of the test's power to alter pre-test probability of an outcome. The likelihood ratio for factor V Leiden testing is 1.5, as shown in Table 18-1. This means that individuals found to have a factor V Leiden mutation are one and a half-times more likely to experience a recurrence than would have been known in the absence of testing. This knowledge does not provide strong clinical guidance.

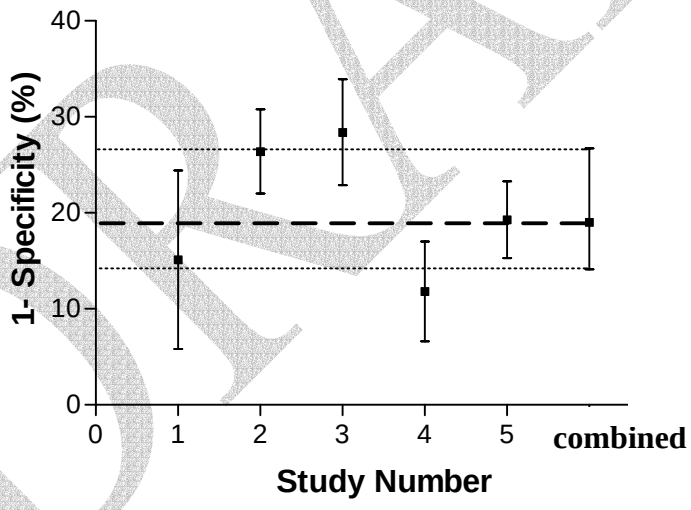

Figure 18-2. Clinical False Positive Rate (1-Specificity) of the Factor V Leiden Mutation Among Individuals Who Will Not Experience a Recurrence. Study numbers are from Table 18-1. The individual and overall mean false positive rates are shown as filled-in squares. Error bars are the 95 percent CI. The combined mean false positive rate is 19 percent (95 percent CI 14-27%). The chi-square for the test for heterogeneity is 36.4 and $p < 0.01$.

Table 18-1. Factor V Leiden -- Clinical Sensitivity, False Positive Rate (1-Specificity), and Likelihood Ratio (LR) for Identifying Recurrent Episodes of Deep Venous Thrombosis

Author	N	N with factor V Leiden	Sensitivity (95 percent CI)	False Positive Rate (95 percent (CI)	LR
1. (Ridker et al., 1995)	77	14	36.4 (10.9-69.2)	15.2 (7.5-26.1)	2.4
2. (Lindmarker et al., 1999)	467	129	35.4 (23.9-48.2)	26.4 (22.1-31.0)	1.3
3. (De Stefano et al., 1999)	395	112	28.3 (20.5-37.3)	28.4 (23.1-34.1)	0.99
4. (Simioni et al., 2000)	224	38	32.7 (20.7-46.7)	11.8 (7.4-17.7)	2.8
5. Unpublished data LETS	474	92	20.0 (11.6-30.8)	19.3 (15.5-23.5)	1.0
Overall	**1637**	**385**	**28** (12.9-34.6)	**19** (14.1-26.7)	**1.5**

Sensitivity and Specificity of Prothrombin G20210A Mutation Testing

Figure 18-3 shows the mean clinical sensitivity and 95 percent confidence interval for the four selected studies of prothrombin G20210A mutation testing. The criteria for selecting these studies were identical to those for the factor V Leiden studies. The asymmetric confidence interval around the estimate for the group of studies is calculated using the DerSimonian and Laird random effects model with an accompanying test of heterogeneity. A total of 1326 individuals was followed for a minimum of 4 years, 95 of whom had a prothrombin G20210A mutation. The overall sensitivity of prothrombin G20210A mutation testing for predicting a recurrence in these studies is 11 percent. The chi-square for the test of heterogeneity in these studies' estimates of the sensitivity is significant. Table 18-2 shows the size of the individual studies, their respective sensitivities and the 95 percent confidence intervals. Details of the studies used for these calculations are in the Appendix. The Appendix also lists studies excluded from the present analyses because study subjects were not fully confirmed to have a first thrombotic event.

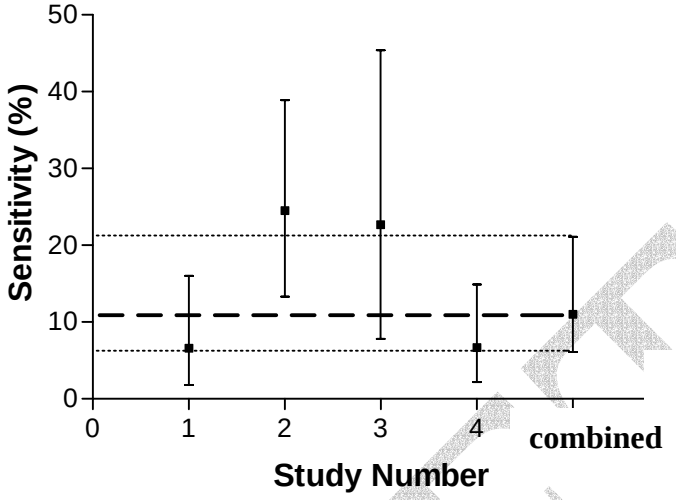

Figure 18-3. Clinical Sensitivity of Prothrombin G20210A Mutation Testing. Study numbers are from Table 18-2. The individual and overall mean sensitivities are shown as filled-in squares. Error Bars are the 95 percent CI. The combined mean sensitivity is 11% (95% CI 6-21%). The chi-square for the test for heterogeneity is 23.3 and p <0.01.

The clinical false positive rate (1-specificity) for prothrombin G20210A mutation testing in the four selected studies is shown in Figure 18-4. The data on which this figure is based are found in Table 18-2. The overall false positive rate is six percent. The chi-square for the test of heterogeneity in these studies' estimates of the false positive rate is significant. Table 18-2 also shows that overall likelihood ratio for prothrombin G20210A testing is 1.8. This is similar to factor V Leiden testing and demonstrates that prothrombin G20210A testing does not provide strong clinical guidance.

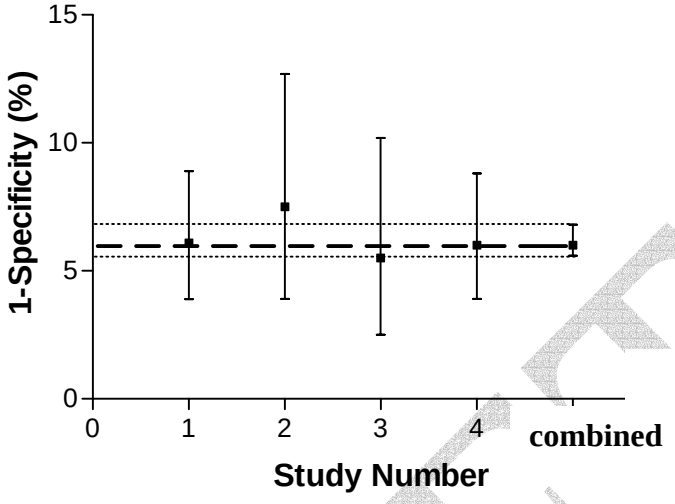

Figure 18-4. Clinical False Positive Rate (1-Specificity) for Prothrombin G20210A Mutation Testing for Individuals Who Will Not Experience a Recurrence. Study numbers are from Table 18-2. The combined mean false positive rate is 6 percent (95 percent CI 5.7-7%). The chi-square for the test for heterogeneity is 5.48 and p <0.01.

Table 18-2. Prothrombin G20210A – Clinical Sensitivity, False Positive Rate (1-Specificity), and Likelihood Ratio (LR) for Identifying Recurrent Episodes of Deep Venous Thrombosis

Author	N	N with Prothrombin G20210A Mutation	Sensitivity (95 percent CI)	False Positive Rate (95 percent CI)	LR
1. (Lindmarker et al., 1999)	456	28	6.6 (1.8-16.0)	6.1 (3.9-8.9)	1.1
2. (Simioni et al., 2000)	210	24	24.5 (13.3-38.9)	7.5 (3.9-12.7)	3.3
3. (Miles et al., 2001)	186	14	22.7 (7.8-45.4)	5.5 (2.5-10.2)	4.1
4. Unpublished data LETS	474	29	6.7 (2.2-14.9)	6.0 (3.9-8.8)	1.1
Summary	**1326**	**95**	**11** (6.2-21.1)	**6** (5.6-6.8)	**1.8**

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APPENDIX

Two-by-Two Tables

The definitions of clinical sensitivity (Question 18) and clinical specificity (Question 19) for predicting a recurrence of venous thrombosis can be derived using a two-by-two contingency table for data from case/control or cohort studies.

Table 1. A Two-by-Two Contingency Table for Deriving the Four Major Clinical Performance Parameters

	Clinically confirmed Recurrence		
	Yes	No	Totals
Factor V Leiden or Prothrombin G20210A Mutation*			
Yes	A	B	A+B
No	C	D	C+D
Totals	A+C	B+D	A+B+C+D

*This example assumes 100% analytic sensitivity and specificity.

- Clinical sensitivity [A / (A + C)] is the proportion of individuals with a confirmed recurrence of venous thrombosis (A+C) who are correctly identified as being factor V Leiden or prothrombin G20210A mutation carriers (A).
- Clinical specificity [D / (B + D)] is the proportion of individuals without a recurrence of venous thrombosis (B+D) who are correctly identified as not being factor V Leiden or prothrombin G20210A mutation carriers (D).
- Positive predictive value [A / (A + B)] is the proportion of positive tests (A + B) that correctly identifies individuals destined to have a recurrence (A). This can be directly derived only if data in the table are from a population-based cohort study(ies).
- Negative predictive value [D / (C + D)] is the proportion of negative tests (C + D) that correctly identifies individuals destined not to have a recurrence (D). This also can be directly derived only if data in the table are from a population-based cohort study(ies).

Test of Heterogeneity

The DerSimonian and Laird modified Cochran method produces a weighted average of differences in rates between studies (Berlin et al., 1989). This method allows for a between-study estimate of variability providing a test for heterogeneity.

Characteristics of Reviewed Studies

Nine studies were found that had information on the effect of the factor V Leiden or prothrombin G20210A mutation for the rate of recurrence. For the calculations described in Questions 18 and 19, we excluded those studies that did not include adequate documentation of a confirmed first

venous thrombotic event, as specified in the disorder definition (Rintelen et al. 1996, Eiching et al. 1997, Margaglione et al. 1999, Kearon et al. 1999, De Stefano 2001). However, all studies are shown in the tables that follow. Overall characteristics of the studies for factor V Leiden calculations are shown in Table 2, and calculations for prothrombin G20210A mutation are shown in Table 5.

The following calculations are performed: sensitivity, specificity, incidence of recurrence per year, and the relative risk (if not specified in the article by the authors). For each article, the yearly incidence of recurrence among factor V Leiden carriers is calculated by dividing the number of individuals with a recurrent event by the number of person-years of follow-up, once for those with, and once for those without, recurrences of venous thrombosis among individuals with a factor V Leiden mutation. The relative risk is the ratio of the two incidence rates.

Factor V Leiden Mutation

Sensitivity and specificity for all studies

Table 2 gives an overall description of the nine available studies. Table 3 shows the sensitivity, false positive rate (1-specificity) and likelihood ratio for all studies. The proportion of factor V Leiden carriers among those with a recurrence ranges from 18.8 to 54.4 percent (sensitivity), with an overall sensitivity of 28 percent for included studies and 26 percent for excluded studies. Table 3 also shows that 11.8 to 48.4 percent of the individuals without a recurrence had the factor V Leiden mutation (false positive rate), with an overall false positive rate of 19 percent for included studies and 27 percent for excluded studies. Table 3 also shows the individual and combined likelihood ratios. The test of heterogeneity for the studies included and excluded from Table 18-1 is also shown. All calculations except sensitivity in included studies involved heterogeneity.

Annual incidence of recurrent episodes of venous thrombosis among factor V Leiden carriers and overall relative risk

Table 4 shows that the mean annual incidence of recurrence in factor V Leiden carriers is 4.8 percent (range 3.0 to 6.7 percent). The relative risk of recurrence for factor V Leiden carriers compared with non-carriers ranges from 1.0 (95 percent CI 0.7-1.5%) to 4.1 (95 percent CI 1.2-14%) in the studies included in the calculations in Table 18-1.

Table 2. Overall Description of Studies Dealing with the Effect of factor V Leiden Mutation on Recurrence, Including the Five Used for Present Calculations and the Four Excluded

Author	age (range)	Country	mean FU years	Type of study	1st Venous Thrombotic Event (n)*	sex M:F	Recurrence (n)
Papers included in calculations for Table 18-1							
Ridker 1995	… (40-84)	USA	5.7	Prospective	77	77:0	11
Lindmarker 1999	58 (15-70)	Sweden	4	Prospective	467	265:202	65
De Stefano 1999	43 (9-77)	Italy	6	Retrospective	395	171:224	120
Simioni 2000	62 (23-84)	Italy	8.3	Prospective	224	117:107	55
Unpublished Data LETS 2002	44 (14-72)	Netherlands	8.1	Prospective	474	272:202	75
Papers excluded in calculations for Table 18-1							
(Rintelen et al., 1996)	49 (21-73)	Austria	5.0	Retrospective	42 (1st or >1)	10:32	11(>1 prev VT)
(Eichinger et al., 1997)	51.1(not given)	Austria	1.7	Prospective	380 (1st or >1)	174:206	36
(Margaglione et al., 1999)	45 (15-85)	Italy	2.8	Retrospective	465(1st or >1)	228:237	63
(Kearon et al., 1999)	>=60	Canada	0.8	Randomized trial	75 (1st or >1)	Not given	16

*This column only includes individuals with factor V Leiden or control individuals, and thus excludes those participants included with a positive prothrombin G20210A test.

Table 3. Factor V Leiden -- Individual and Collective Clinical Sensitivities, False Positive Rates (1-Specificity), and Likelihood Ratios Found by the Nine Studies in Table 2 for Predicting a Recurrence

Author	N	N with factor V Leiden	Sensitivity (95%CI)	False Positive Rate (1-Specificity) (95%CI)	LR*
Papers included in calculations Table 18-1					
Ridker 1995	77	14	36.4 (10.9-69.2)	15.2 (7.5-26.1)	2.4
Lindmarker 1999	467	129	35.4 (23.9-48.2)	26.4 (22.1-31.0)	1.3
De Stefano 1999	395	112	28.3 (20.5-37.3)	28.4 (23.1-34.1)	0.99
Simioni 2000	224	38	32.7 (20.7-46.7)	11.8 (7.4-17.7)	2.8
Unpublished data LETS	474	92	20.0 (11.6-30.8)	19.3 (15.5-23.5)	1.0
Overall	**1637**	**385**	**28** (12.9-34.6)[1]	**19** (14.1-26.7)[2]	**1.5**
Papers excluded in calculations Table 18-1					
Rintelen 1996	42	21	54.5(23.4-83.2)	48.4 (30.2-66.9)	1.1
Eichinger 1997	380	112	27.8 (14.2-45.2)	29.7 (24.9-34.8)	0.9
Margaglione 1999	465	99	25.4 (15.3-37.9)	20.6 (16.8-24.9)	1.2
Kearon 1999	75	20	18.8 (4.0-45.6)	28.8 (17.8-42.1)	0.6
Overall	**962**	**252**	**26** (18.3-37.8)[3]	**27** (20.1-36.3)[4]	**0.9**

*Likelihood ratio

1- Test of heterogeneity: χ^2=3.43, p>0.05

2- Test of heterogeneity: χ^2=36.4, p<0.01

3- Test of heterogeneity: χ^2=1.66, p>0.05

4- Test of heterogeneity: χ^2=9.03, p<0.01

Table 4. Annual Incidences and Relative Risks of Recurrence Among factor V Leiden Carriers found by the Nine Studies in Table 2

Author	Factor V Leiden mutation (N)	Factor V Leiden carrier frequency %‡	Factor V Leiden mutation with Recurrence (N)	Incidence of Recurrence in factor V Leiden carriers per year %	Overall RR (95%CI) (with factor V Leiden vs. without factor V Leiden)
Papers included in calculations					
Ridker 1995	14(0)**	18.2	4(0)**	5.0	RR 4.1 [1.2-14]
Lindmarker 1999	129(11)	27.6	23(4)	4.5	RR=1.4 [0.8-2.3]
De Stefano 1999	112(0)	28.4	34(0)	5.0	RR 1.1 [0.7-1.6]
Simioni 2000	38(0)	17.0	18(0)	6.7	RR: 2.4 [1.4-4.1]
Unpublished data LETS	92 (8)	19.4	15 (not given)	3.0	RR: 1.0 [0.7-1.5]
Summary				**4.8**	
Papers excluded in calculations					
Rintelen 1996	21(5)	50.0	6(2)	5.7	RR=1.0 [0.3-3.3]
Eichinger 1997	112(10)	29.5	10(1)	5.2	RR=1.0 [0.5-2.1]
Margaglione 1999	99(not given)	21.3	16 (not given)	5.8	OR: 1.3 [0.7-2.4]
Kearon 1999	20(1)	26.7	3 (1)	19.0	RR: 0.5 [0.1-1.8]

‡ Carrier frequency of the factor V Leiden mutation among individuals with a first venous thrombosis
**Number in ( ) = Homozygotes

Prothrombin G20210A mutation

Clinical Sensitivity and specificity for all studies

Table 5 shows the important characteristics of the seven available studies. Table 6 shows the sensitivity, false positive rate (1-specificity) and likelihood ratio for all studies. The prevalence of prothrombin G20210A carriers among those with a recurrence ranged in different studies from 6.6 to 24.5 percent (clinical sensitivity), with an overall clinical sensitivity of 11 percent for included studies and 12 percent for excluded studies. The prevalence of individuals without the prothrombin G20210A mutation among those with a first but not a second episode of venous thrombosis ranged in these studies from 85 percent to 94.5 percent. This means that 5.5 to 15 percent of the individuals without a recurrence had the prothrombin G20210A mutation (false positive rate), with an overall false positive rate of 6 percent for included studies and 12 percent for excluded studies. Table 3 also shows the individual and combined likelihood ratios. The test of heterogeneity for the studies included and excluded from Table 18-2 is shown. All calculations involved heterogeneity.

Incidence of recurrence in prothrombin G20210A carriers and overall relative risk

Table 7 shows that the mean annual incidence of recurrence in prothrombin G20210A carriers was 4.2 percent (range 2.1 to 6.0 percent) and that the relative risk of recurrence for prothrombin G20210A carriers compared with non-carriers ranged from 0.9 percent (95 percent CI 0.2-2.9%) to 4.9 percent (95 percent CI 1.9-12.9%) in studies included in the calculations included in Table 18-2.

Table 5. Overall Description of Studies Dealing with the Effect of Prothrombin G20210A on Recurrence, Including the Four Used for Present Calculations and the Three Excluded

Author	age (range)	Country	mean follow-up (years)	Type of study	1st venous thrombotic event (n)*	sex M:F	Recurrence (n)
Papers included in calculations							
Lindmarker 1999	58(15-70)	Sweden	4	Prospective follow-up	456	Not given	61
Simioni 2000	62(23-84)	Italy	8.3	Prospective follow-up	210	106:104	49
Miles 2001	(see previous article)	USA	7.3	Prospective follow-up	186	186:0	22**
Unpublished Data - LETS	44 (14-72)	Netherlands	8.1	Prospective follow-up	474	272:202	75
Papers excluded in calculations							
Eichinger 1999	49.8 (not given)	Austria	2.0	Prospective follow-up	492 (1st or >1)	227/265	57(>1 prev VT)
Margaglione 1999	45(15-85)	Italy	2.8	Retrospective follow-up	421 (1st or >1)	197:224	58
(De Stefano et al., 2001)	43.7	Italy	6	Retrospective follow-up	335 (1st or >1)	146:189	115

*This column only includes individuals with the prothrombin G20210A mutation or control individuals, and thus excludes those participants included with a positive factor V Leiden test. However, for the study by Eichinger et al 1999, Miles et al 2001 it was not possible to exclude the factor V Leiden positive individuals from the calculations.

**10% of all non-carriers (n=172) had a recurrence, so the number of recurrences could be 17 or 18, and therefore the total number 22 or 23

Table 6. Prothrombin G20210A Mutation -- Clinical Sensitivity, False Positive Rate (1-Specificity), and Likelihood Ratio for Recurrence

Author	**N**	**N with Prothrombin G20210A Mutation**	**Sensitivity (95%CI)**	False Positive Rate (95%CI)	LR
Papers included in calculations in Table 18-2					
Lindmarker 1999	456	28	6.6 (1.8-16.0)	6.1 (3.9-8.9)	1.1
Simioni 2000	210	24	24.5 (13.3-38.9)	7.5 (3.9-12.7)	3.3
Miles 2001	186	14	22.7 (7.8-45.4)	5.5 (2.5-10.2)	4.1
Unpublished data LETS	474	29	6.7 (2.2-14.9)	6.0 (3.9-8.8)	1.1
Summary	**1326**	**95**	**11** (6.2-21.1)[1]	**6** (5.6-6.8)[2]	**1.8**
Papers excluded in calculations in Table 18-2					
Eichinger 1999	492	42	9.4 (1.1-14.6)	8.1 (6.4-12.0)	1.2
Margaglione 1999	421	55	19.0 (9.9-31.4)	12.1 (9.0-15.4)	1.6
De Sefano 2001	335	52	16.5 (12.5-24.6)	15.0 (10.6-20.4)	1.1
Summary	**1248**	**149**	**12** (5.1-26.9)[3]	**12** (8.7-15.6)[4]	**1.2**

1- Test of heterogeneity: χ^2=23.5, p<0.01
2- Test of heterogeneity: χ^2=35.8, p<0.01
3- Test of heterogeneity: χ^2=5.48, p<0.05
4- Test of heterogeneity: χ^2=18.8, p<0.01

Table 7. Annual Incidences and Relative Risks of Recurrence Among Prothrombin G20210A Carriers Found by the Seven Studies in Table 5

Author	Prothrombin G20210A mutation (N)	Prothrombin G20210A mutation carrier frequency %‡	Heterozygous Prothrombin G20210A mutation with recurrence (N)	Incidence of Recurrence in Prothrombin G20210A mutation carriers per year %	overall RR (95%CI) (with prothrombin G20210A mutation vs. without prothrombin G20210A mutation)
Papers included in calculations					
Lindmarker 1999	28 (0)**	6.1	4 (0)**	3.6	OR: 0.9 [0.2-2.9]
Simioni 2000	24 (0)	11.4	12 (0)	6.0	RR: 2.4 [1.3-4.7]
Miles 2001*	14 (0)	7.5	5 (0)	4.9	RR: 4.9 [1.9-12.9]
Unpublished data LETS	29 (0)	6.1	5 (0)	2.1	RR: 1.1 [0.5-2.5]
Summary				**4.2**	
Papers excluded in calculations					
Eichinger 1999*	42 (1)	8.5	3 (not given)	3.6	RR: 0.7 [0.2-2.1]†
Margaglione 1999	55 (not given)	13.1	11 (not given)	7.1	OR: 1.7 [0.8-3.5]
De Stefano 2001	52 (0)	15.5	19 (0)	5.2^	RR: 1.2 [0.7-1.9]

* In the articles by Eichinger et al 1999 and Miles et al 2001 it was not possible to exclude individuals who carried the factor V Leiden mutation.

^ Mean number of follow-up years was 7 in the prothrombin G20210A mutation carriers.

‡ Carrier frequency of the prothrombin G20210A mutation in patients with a first venous thrombosis event

† RR was adjusted for the factor V Leiden carrier status and age

**Numbers in ( ) = Homozygotes

General discussion

Possible explanations for the large range of incidences per year and relative risks among different studies are shown in Table 8.

Table 8. Possible differences among studies

- Differences in inception cohorts
- Differences in inclusion criteria
- Location of initial clot
- Exclusion of pulmonary thrombosis
- Differences in treatments
- Presence of other thrombotic risk modifiers
- Small sample size

The source of the patients could affect the incidence rate of recurrence found in these studies. Having other thrombotic risk factors increases the risk. Thus, in thrombosis referral centers the chance of finding individuals with multiple risk factors is higher. These centers will be more likely to have individuals with familial thrombophilia who have an increased risk of venous thrombosis compared with unselected patients (Lensen et al. 2000). However, although three studies (Rintelen et al 1996, De Stefano et al 1999, De Stefano et al 2001) included individuals from thrombosis referral centers, these studies found approximately the same annual incidence of recurrence as found in the other studies. Some studies on the association of factor V Leiden and the risk of recurrence were performed before or during the year of discovery of the prothrombin G20210A mutation (1996). In these papers, the rate of recurrence could be affected, due to the unknown distribution of the prothrombin G20210A mutation in the study groups. The high recurrence rate found by Kearon is an outlier. No explanation for the high risk found in this study could be found in the paper, or from personal communications with the lead author. None of the studies mentioned different treatment strategies for individuals with or without the factor V Leiden or the prothrombin G20210A mutation. Therefore, it is unlikely that this affected the risk estimates reported in these studies.

Details on these issues are summarized per article below

Notes on Studies Involving factor V Leiden or Prothrombin 20210A Selected for Inclusion in the Present Analysis (in order of appearance in Tables)

Ridker et al 1995

Inception cohort: US male physicians who were originally included for a randomized trial on low dose aspirin (n=22071). Seventy-seven of the 14916 males who provided baseline specimens had a first objectively confirmed thrombotic event (deep venous thrombosis or pulmonary embolism) and were followed prospectively.

Inclusion criteria: Individuals with idiopathic events (i.e. not occurring during surgery or cancer).

Use of OAC: All events occurred after discontinuation of OAC. Duration of OAC was according to standard outpatient anticoagulation regimens (IV heparin 5-7 days, followed by 3 months of Warfarin).

Modifiers: Adjusted estimates of risk were computed that controlled for age and smoking habit. No association was found between FVL and BMI, exercise, hypertension or diabetes.

Comments: Although the incidence of recurrence per year in FVL carriers in this study is similar to most other studies, the relative risk of a recurrent venous thrombosis in FVL carriers versus non-carriers is the highest of all studies (RR=4.1, with a wide confidence interval of 1.2-14.0 due to a low number of participants). This could only be explained by a very low incidence of recurrence in non-carriers, which could be due to the selection of male physicians who might be healthier than males from the normal population.

Lindmarker et al 1999

Inception cohort: Patients were originally included for a randomized multi center trial (Duration of anticoagulation study; DURAC trial) to compare the effect of 6 weeks OAC with 6 months OAC treatment after a first VTE event.

Inclusion criteria: Age <71 years, no other anticoagulant defects. Exclusion was for major bleeding event, oral anti-vitamin K therapy for other than VTE indications and thromboprophylaxis during pregnancy.

Use of OAC: 50.7% had 6 weeks of oral anticoagulant therapy following the first event instead of 6 months.

Modifiers: Relative risks were adjusted for sex and age. Age, sex, thrombus site and idiopathic thrombosis (or a permanent risk factor) were all statistically significantly associated with a recurrent event within 48 months.

Comments: There is an unknown gap between the first event and the start of this study. The RR for the risk of a recurrent event after 6 weeks versus 6 months of therapy was 1.6 (0.9-2.9). No details are given on the distribution of individuals (FVL carriers or non-carriers) within the 6 weeks or 6 months therapy groups.

De Stefano et al 1999

Inception cohort: Patients with a first episode of deep venous thrombosis of the legs who were referred to two specialized thrombosis centers for an assessment of the possible causes of thrombophilia.

Inclusion criteria: No other anticoagulant defects, cancer or myeloproliferative diseases, autoimmune disorder and treatment with an oral anticoagulant for more than six months after the first episode of deep venous thrombosis, or a diagnosis of recurrent superficial venous thrombosis without objective signs of deep venous thrombosis.

Use of OAC: Not all patients followed the recommendations to not take oral contraceptives and to start antithrombotic prophylaxis during risk situation. It is unknown whether events occurred during anticoagulation treatment.

Modifiers: Stratification of the risk of recurrence among patients who were heterozygous for factor V Leiden alone as compared with those with neither mutation according to whether the first event or recurrent event was spontaneous or due to known risk factor did not change the results substantially. Analysis of the time-to-recurrence curves showed that the presence of factor V Leiden did not significantly increase the risk of recurrent deep venous thrombosis among men or women.

Comments: The time between age at first episode of DVT and age at referral was approximately 8 years. The inception cohort consisted of individuals with one or more previous thrombotic events referred to a thrombosis center.

Simioni et al 2000

Inception cohort: Outpatients with a first venography-proven DVT were included and prospectively followed.

Inclusion criteria: Absence of malignant disease or an abnormality in the coagulation or fibrinolytic system. Also patients who needed vitamin K antagonist therapy for reasons other than venous thromboembolism were excluded.

Use of OAC: All patients were treated with anticoagulants for a period of 3 to 6 months independent of carrier status. Some recurrences occurred, according to a previous article from 1997 on this cohort, during oral anticoagulation therapy (2.9% in individuals without FVL and 9.8% in individuals with FVL).

Modifiers: Adjustment for age and cause of the event (idiopathic or induced) did not change the risk estimate.

Unpublished data LETS 2002

Inception cohort: In this study, all patients with a first objectively diagnosed episode of DVT were included and followed.

Inclusion criteria: Patients without malignant diseases, aged < 70 years.

Use of OAC: All patients were treated according to the normal standards in the Netherlands

Modifiers: Not looked at yet.

Miles et al 2001

Inception cohort: This study uses the cohort of US male physicians that were originally included for a randomized trial on the effect of low dose aspirin (n=22071). Two-hundred and eighteen men with venous thromboembolism were followed prospectively.

Inclusion criteria: No other inclusion criteria than the above.

Use of OAC: All events occurred after discontinuation of OAC.
Modifiers: Adjusted estimates of risk were computed that controlled for age, smoking and BMI. The risk for recurrent VTE was similar in those with idiopathic (i.e. not related to cancer, trauma or surgery) initial venous thromboembolism.

Use of OAC: None received anticoagulant treatment at time of inclusion and experienced a recurrent event during anticoagulation treatment.
Modifiers: Controls are age and sex-matched.
Comments: The first event was not a recent event; the current median age was much higher than the age at first event (± 15 years difference). In addition, the age at first event was lower in FVL homozygotes and more additional risk situations were present in control patients. This very small inception cohort consisted of individuals referred to a thrombosis center.

Notes on Studies Involving factor V Leiden or Prothrombin 20210A NOT Selected for Inclusion in the Present Analysis (in order of appearance in Tables)

Rintelen et al 1996

Inception cohort: Patients with a history of a first or recurrent venous thrombosis (indicating one or more events!). An attempt has been made to divide these patients into patients with one earlier event and patients with more than one earlier event. 19 patients have only one earlier event (7 heterozygous FVL, 12 without FVL). Only 2 of these patients had a recurrent event, both did not have FVL. All these patients were referred to thrombosis centers. The design of the study is retrospective with an observation time up to 108 person-years.

Inclusion criteria: Young age at first thrombosis, recurrence of thrombosis or a positive family history.

Eichinger et al 1997

Inception cohort: Consecutive individuals with a first or recurrent VTE.
A separation could be made from the table in individuals with a first VTE at study entry and individuals with recurrent VTE at study entry. Thirteen percent of the individuals with a first event at study entry with the factor V Leiden mutation had a recurrence compared with 14 percent of the individuals with a first event at study entry without the factor V Leiden mutation. However, as no observation time was given for individuals with a first event at study entry only, the incidence of the recurrence rate per year and the relative risk could not be calculated. The risk of recurrent VTE was similar in patients with recurrent VTE at study entry and patients with a first VTE at study entry.

Inclusion criteria: Age older than 18 years, absence of a known anticoagulation defect, absence of cancer, no other reasons for long-term treatment with antithrombotic drugs.

Use of OAC: Inclusion was after discontinuation of OAC and no events during follow-up occurred during anticoagulation treatment. Treatment was for at least three months.

Modifiers: Patients with and without factor V Leiden with recurrent events were comparable to patients without recurrences with regard to sex, age, presence of a positive family history, and the number of previous thromboembolic events.

Comments: Approximately 3 years' difference between 1st event and study entry. The inception cohort consisted of individuals with one or more previous thrombotic events.

Margaglione et al 1999

Inception cohort: Subjects with a documented first or recurrent venous thrombosis in one leg, who had been referred for a work-up were investigated retrospectively.

Inclusion criteria: No particular inclusion criteria beside the above criteria.

Use of OAC: All patients had been treated with oral anticoagulant therapy for a period of at least six months. One patient suffered from a recurrent episode of venous thromboembolism during oral anticoagulant therapy.

Modifiers: Odds ratios were adjusted for age, sex and presence of history of arterial thrombotic episodes.

Comments: The median time elapsed from thrombosis to blood collection was 8 months, but nothing is said on the age at first thrombosis. The inception cohort consisted of individuals with one or more previous thrombotic events.

Kearon et al 1999

Inception cohort: Patients who have a first episode of venous thromboembolism in the absence of a major thrombogenic risk factor (idiopathic thrombosis) were included in a randomized trial on anticoagulation. Results presented here are on the placebo group.

Inclusion criteria: Ineligible if they had other indications for or a contraindication to long-term anticoagulant therapy, required long-term treatment with other medicinal drugs like non-steroidal anti-inflammatory drugs, a familial bleeding diathesis, a major psychiatric disorder, are pregnant or could become pregnant, were allergic to contrast medium, had a life expectancy of less than two years, were initially treated with a non-licensed preparation of low-molecular-weight heparin, were considered likely to be noncompliant, or were unable to complete follow-up visits because of the distance from their residence to the medical center.

Use of OAC: All patients have completed 3 months of anticoagulation before study entry. After consent, randomization was performed for warfarin or identical-appearing placebo.

Modifiers: The presence of a lupus anticoagulant was the only variable significantly associated with recurrent venous thromboembolism.

Comments: Although the authors included patients with an idiopathic thrombosis, they did not screen for biochemical risk factors and did include patients with episodes that were secondary to a transient risk factor. The inception cohort was very small and the age of the participants was 60 years or older.

Eichinger et al 1999

Inception cohort: In this study the risk of recurrent VTE was assessed in consecutive individuals with a first or recurrent VTE. A separation could be made from the table in individuals with a first VTE at study entry

and individuals with recurrent VTE at study entry. The number of individuals who had one venous thrombosis at study entry was 393. Not enough information was available to make the calculations for this particular group separately.

Inclusion criteria: Age older than 18 years, absence of a known anticoagulation defect, absence of cancer, no other reasons for long-term treatment with antithrombotic drugs.

Use of OAC: Inclusion was after discontinuation of OAC and no events during follow-up occurred during anticoagulation treatment. Treatment was for at least three months.

Modifiers: Patients with and without factor V Leiden with recurrent events were comparable to patients without recurrences with regard to sex, age, presence of a positive family history, and the number of previous thromboembolic events.

De Stefano et al 2001

Inception cohort: Patients with a history of one or more episodes of deep vein thrombosis of the legs who were referred to two specialized thrombosis centers.

Inclusion criteria: Those with normal thrombophilia screening or carriers of the G20210A mutation. Exclusion if other thrombotic defects, cancer or myeloproliferative diseases, autoimmune disorders, or longer than 6 months or oral anticoagulant therapy after the first deep vein thrombosis.

Use of OAC: Information on use of OAC is missing.

Modifiers: Stratification of recurrences according to the circumstances of the first event (spontaneous or secondary) produced no substantial difference. The rate of recurrence was similar in men and in women.