Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation

Jan P Vandenbroucke, Ted Koster, Ernest Briët, Pieter H Reitsma, Rogier M Bertina, Frits R Rosendaal

Summary
We investigated whether the occurrence of venous thrombosis in young women who use oral contraceptives might be explained by the factor V Leiden mutation, which leads to resistance to activated protein C and enhances susceptibility to thrombosis.

We compared 155 consecutive premenopausal women, aged 15 to 49, who had developed deep venous thrombosis in the absence of other underlying diseases, with 169 population controls. The risk of thrombosis among users of oral contraceptives was increased 4-fold (relative risk 3.8 [95% CI 2.4-6.0]). The risk of thrombosis among carriers of the mutation compared with non-carriers was increased 8-fold (7.9 [3.2-19.4]). Compared with women who did not use oral contraceptives and were not carriers of the mutation, the risk of thrombosis among those with both risk factors was increased more than 30-fold (34.7 [7.8-154]).

Recalculation of population incidences from these relative risks shows that the absolute risk of venous thrombosis in young women who use oral contraceptives is much larger when they carry the factor V Leiden mutation.

When a young woman develops thrombosis, her factor V Leiden Status should be considered in counselling about her future method of contraception.

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Introduction
Ever since the first report in 1961,1 the risk of venous thrombosis has been a much debated hazard of the use of oral contraceptives.2-3 Venous thrombosis has become less common but has not disappeared with present-day low-dose preparations.4-6 It is still unclear why oral contraceptives cause thrombosis, and why they do so only in a small number of women.

A hereditary abnormality in the protein C/protein S anticoagulant pathway, which leads to increased susceptibility to venous thrombosis, has been identified lately.7 The abnormality is resistance to the anticoagulant effect of activated protein C (APC-resistance). Activated protein C inhibits clotting by cleavage of factors Va and Vila, and APC-resistance is caused by a point mutation at a cleavage site in factor Va which makes it inaccessible to activated protein C.8 The mutation, a G/A substitution at nucleotide position 1691 of the factor V gene which has been called factor V Leiden,9 has been confirmed by others.10 It corresponds strictly with the presence of hereditary APC-resistance.11

We found APC-resistance in about 20% of patients with venous thrombosis and 3-5% of a general population sample in the Netherlands.10,11 In a Swedish study, APC-resistance was present in 33% of thrombosis patients and 5% of controls.12 These findings showed that APC-resistance is a strong risk factor for venous thrombosis and that it is much more prevalent than the other known hereditary abnormalities of the anticoagulant pathway that lead to increased risk of venous thrombosis (ie, protein C, protein S, and antithrombin deficiency).

We have investigated whether the presence of the factor V Leiden mutation could explain thrombosis in young women who use oral contraceptives. Specifically, we wanted to know whether the risk of thrombosis that exists in oral-contraceptive users is increased in those who carry the mutation. The investigation was part of a population-based case-control study on hereditary factors in venous thrombosis, the Leiden Thrombophilia Study.13

Patients and methods
The design of our population-based case-control study has been described previously.13 Consecutive patients younger than 70, with an objective diagnosis of a first episode of deep venous thrombosis between 1988 and 1993 and without an underlying malignant disorder, were selected from the files of three anticoagulation clinics in the Netherlands which monitor anticoagulation treatment of virtually all patients in three well-
disease in the Netherlands can be estimated over the 5 years of our study as 2 in 10000 woman-years (17/109 824) which has a geographical source population of 109 824 m. We estimated the total number of person-years that had yielded visuahsanon of the cleavage products on ethidium-bromide-stained agarose gels. We carried out logistic regression to assess the direction and magnitude of the multiplicative interaction between recent use of oral contraceptives and the factor V Leiden mutation and to evaluate the effect of controlling for the matching factor age and the effects of other potential risk factors such as smoking, obesity (Quetelet index greater than 30 kg/m²), surgery in the month before the thrombosis index date, and diabetes.

### Results

109 (70%) of the 155 women with first venous thrombosis and no other underlying disorder had been using oral contraceptives in the month before thrombosis compared with 65 (38%) of the 169 controls. This amounts to an increase in risk (relative risk) of 3.8 (95% CI 2.4–6.0) of the 155 women with thrombosis, 35 (23%) carried the factor V Leiden mutation (5 homozygotes and 30 heterozygotes) compared with 6 (3.6%) controls (all heterozygotes). This leads to an increase in risk of venous thrombosis among carriers of the factor V Leiden mutation of 7.9 (3.2–19.4). When the analysis was restricted to heterozygotes, the relative risk was 6.8 (2.7–16.8).

The relative risk of thrombosis associated with oral contraceptives was at least as high in carriers of the mutation as in women with the normal genotype (table 1, 5.0 vs 3.7). This result was similar after exclusion of the homozygotes from the analysis (5 women, of whom 3 used oral contraceptives, relative risk 5.5 [0.8–36]). The small difference in relative risk between carriers of the mutation and women with the normal genotype is statistically uncertain, however, owing to the small number of control women with the factor V Leiden mutation (only 6 women). As a first approximation, therefore, we conclude that the relative risk of thrombosis due to current oral-contraceptive use is similar among carriers and non-carriers of the mutation and does not significantly differ from the overall 4-fold relative risk associated with oral-contraceptive use. However, the presence of the mutation itself causes a 7–8-fold increase in risk. This increase is also found for women in the absence of oral-contraceptive use, of the patients who did not use the pill, 10 carried the mutation and 36 did not compared with 4 and 100 controls, a relative risk of 7.0 (2.1–23.5) for the factor V Leiden mutation in the absence of oral contraceptives. The additional use of oral contraceptives will multiply this 7-fold increase by 4, so that a woman who uses oral contraceptives and is a carrier of the mutation will have a risk of venous thrombosis about 30 times that of a non-user who does not carry the mutation. This can also be calculated directly from table 1 by contrasting the relative frequency of having both risk factors versus none among patients and controls of the patients, 25 had both risk factors and 36 none, compared with 2 and 100 among the controls (relative risk 34.7 [7.8–154]). Since we know that first thrombosis in the absence of underlying disease has an approximate incidence of 2 in 10000 woman-years (17/109 824) we expect this attenuation to be slight. We also expect little attenuation of the effect of the factor V Leiden mutation, since its prevalence is likely to be independent of age, except for sample fluctuation, because it is an autosomal genetic abnormality.

We used logistic regression to assess the direction and magnitude of the multiplicative interaction between recent use of oral contraceptives and the factor V Leiden mutation and to evaluate the effect of controlling for the matching factor age and the effects of other potential risk factors such as smoking, obesity (Quetelet index greater than 30 kg/m²), surgery in the month before the thrombosis index date, and diabetes.

### Table 1 Current use of oral contraceptives among patients and controls according to presence of factor V Leiden mutation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Controls</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current OC use</td>
<td>25</td>
<td>2</td>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td>No current OC use</td>
<td>10</td>
<td>4</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>5.0 (0.8–31.8)</td>
<td>3.7 (2.2–6.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although the original data were age matched, we present analyses on unmatched data. Undoung the matching permits analysis of subgroups of cases and controls selected for variables upon which they were not matched. It also enables the presentation of the raw data. A disadvantage is that the unmatched analysis will diminish the contrast between the groups, especially with respect to the effect of oral contraceptives. Since, however, our analysis was already restricted to one of the matching factors (sex) and partly restricted to the other (age), we expect this attenuation to be slight. We also expect little attenuation of the effect of the factor V Leiden mutation, since its prevalence is likely to be independent of age, except for sample fluctuation, because it is an autosomal genetic abnormality.

Relative risks of thrombosis among current users of oral contraceptives were estimated as odds ratios and their 95% CI according to Woolf. To show the absolute effect of the cumulation of risk factors, we also estimated the population incidence of thrombosis in young women with the four possible combinations of factor V mutation and use of oral contraceptives. We estimated the total number of person-years that had yielded the cases and partitioned these person-years according to the distribution of pill use and the factor V mutation in the control group. Since we know that in our original study 117 female patients aged 15–49 came from the Leiden anticoagulation clinic, which has a geographical source population of 109 824 in that age group (data provided by the municipal administration), first thrombosis incidence among young women without underlying disease in the Netherlands can be estimated over the 5 years of our study as 2 in 100 000 woman-years (117/5×109 824).
effect of oral contraceptive use and factor V Leiden interaction The analysis confirmed a near-multiplicative
mutated gene, the relative risk became 58 (32-105), 81 with a multiplicative interaction
contraceptives 60 (34-106) and factor V Leiden
small independent effect As expected, statistical
with diabetes and recent surgery (2 and 17) led to
deep venous thrombosis The small numbers of women
match the cumulative relative risk of homozygotes to more
so even a smaller increase in thrombosis risk by
homzygotes is more than 100-fold " A difficulty in the
interaction parameters is more than additive
increase the number of cases by 22.8 per 10 000 women
without the mutation, use of oral contraceptives yields an
V Leiden carriers than women who do not In women
without the mutation, use of oral contraceptives yields an
additional 2.2 cases per 10 000 women per year, whereas
among carriers of the mutation, oral contraceptives increase the number of cases by 2.2 times per 10 000 women per year. This finding indicates that the joint effect of the two risk factors is more than additive.

The increased risk is seen when heterozygotes and
homozygotes are taken together and is the same for
heterozygotes only (the majority of the patients with the
mutation). All 5 homozygotes were patients with deep
venous thrombosis and 3 were current users of oral
contraceptives. However, the numbers are small, this
proportion of oral-contraceptive use matches that in the
control group, since it is unlikely that a hitherto unknown
factor V Leiden mutation strongly
increases the risk of venous thromboembolism. The combined
risk due to oral contraception is about 0.8 per 10000
women per year, whereas among carriers of the mutation, the
risk increase for oral-contraceptive users who also carry the genetic
factor is more than 100-fold. The increased risk due to oral
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risk increase for oral-contraceptive users who also carry the genetic
factor is more than 100-fold. Therefore, a more stable estimate can be obtained by

<table>
<thead>
<tr>
<th>Patients</th>
<th>Person-years</th>
<th>Incidence per 10 000 person-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No OC use</td>
<td>36</td>
<td>437 870</td>
</tr>
<tr>
<td>Current OC use</td>
<td>84</td>
<td>275 858</td>
</tr>
<tr>
<td>Factor V Leiden positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No OC use</td>
<td>10</td>
<td>17 515</td>
</tr>
<tr>
<td>Current OC use</td>
<td>25</td>
<td>8757</td>
</tr>
</tbody>
</table>

* A total of 740 000 person-years (yielding 155 patients) was partitioned according to
the distribution of the control group 100:63:4/2

Table 2 Estimated population incidence of first venous
thrombosis in women aged 15-49, according to presence of
factor V Leiden mutation and use of oral contraceptives

10 000 person-years in this age group, we can calculate that the 155 cases originate from a population of about
740 000 person-years. The distribution of these person-years over current use of oral contraceptives and factor V
Leiden carriage can be derived from the control group, which is representative of the population from which these
cases originated. The incidence of thrombosis increases from 0.8 to 10 000 women per year for non-users of oral
contraceptives without the mutation to 28.5 per 10 000 women with the mutation who also use oral contraceptives (table 2). The absolute increase in
thrombosis risk due to oral contraceptives (ie, the risk difference) is much larger in women who carry the factor V
Leiden mutation than in women who do not. In women
without the mutation, use of oral contraceptives yields an
additional 2.2 cases per 10 000 women per year, whereas
among carriers of the mutation oral contraceptives increase the number of cases by 2.2 times per 10 000 women per year. This finding indicates that the joint effect of the two risk factors is more than additive.
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**Discussion**
The combination of the use of oral contraceptives and
and factor V Leiden carriage of the factor V Leiden mutation strongly
increases the risk of venous thromboembolism. The combined
effect of these risk factors seems close to a multiplica-
tion of the separate relative risks. In terms of absolute
risk, however, this means that the risk of venous
thromboembolism among women who use oral contraceptives is
much greater when they carry the factor V Leiden
mutation.

Carriage of the factor V Leiden mutation and APC-
resistance led to a 7-8-fold increase in venous thrombosis
risk in our study in the Netherlands. The Swedish
estimate was somewhat higher (10 [4.0-25.0], recalculated from Svensson and Dahlback). The difference is possibly due to differences in patient samples—a proportion of the patients in the Swedish study had recurrent or familial thrombosis.

Current oral-contraceptive use in our study gave a close
to 4-fold risk, which increased to 6-fold after adjustment
for age. These estimates are similar to those of the few
studies that have specifically addressed the risk of venous
thromboembolism among women using modern low-dose oral
contraceptives, which were also predominant in the
Netherlands during our study period. The Oxford Family
Planning Study found a relative risk of 6.5 (1.2-16.2), recalculated, the Puget Sound study recalculated, the Puget Sound study 2.8 (0.9-8.2), and a study in Boston 8.1 (3.7-18), with no indication of a
decreased risk in the lowest dose group. The confidence
intervals from these studies are wide and overlapping,
because of the small numbers. Older studies on high-dose
pills had relative risk estimates between 2 and 11. Several of these older investigations used only a classical
diagnosis of venous thromboembolism, which is now known to
be highly unreliable with misclassification rates of up to
50%. In studies with comparable diagnostic criteria, however, low-dose preparations confer less risk than the
higher-dose pills. In our study the multiplication in risk caused by oral
contraceptives was of similar magnitude in carriers and
non-carriers of the factor V Leiden mutation. Therefore
our present best estimate is a cumulative 30-fold increase for oral-contraceptive users who also carry the genetic
risk. By a similar multiplicative effect, the risk increase for
homozygotes is more than 100-fold. A difficulty in the
interpretation of our data is that the confidence interval of
the relative risk of oral-contraceptive use among carriers of
the factor V Leiden mutation is large, mainly because
of the small number of women without thrombosis who
carry the mutation (table 1). To obtain a more reliable
estimate we would need a study with larger numbers of
controls positive for factor V Leiden. Since the prevalence
of factor V Leiden in the general population is 3-5%, a
case-control study several times larger than this one
would be needed. However, we can offer two other and
different approximations to estimate the use of oral
contraceptives among women who carry the mutation. The proportion of such women using oral contraceptives
should not differ too much from that in the general
population, since it is unlikely that a hitherto unknown
mutation would influence the use of contraceptives,
especially in women who have not yet had a thrombosis.
Therefore, a more stable estimate can be obtained by

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the clotting cascade due to oral contraceptives probably
mutation (APC-resistance) and the metabolic changes in
relative risks are exactly multiplicative, it is the additional
reasoning it is not important to know whether or not the
either risk factor alone
They developed thromboses
which is much larger than the sum of the individual
effects Thus, in the presence of both risk factors, venous
thrombosis is low even among young women who have
both risk factors. Most episodes among the young are
minor, although pulmonary embolism does occur
Withholding oral contraceptives from all carriers might be
a high price to pay, especially since other methods of
contraception are more error-prone and cause greater
medical, psychological, and social morbidity. Although it
might be tempting to screen for homozygotes, who have a
greatly increased risk, such an undertaking would not be
cost-effective since the population frequency of
homozygosity of the factor V Leiden mutation is only
about 1 in 5000

When a young woman has had venous thrombosis,
however, her factor V Leiden status might be take into
account in counseling about future methods of
contraception. If she is a homozygote, she should be
strongly advised to discontinue oral contraception. If she
is a heterozygote, the increased risk should be clearly
explained, since her thrombosis episode shows that she
might be one of the women who are susceptible to the
interplay between oral contraceptives and hereditary
APC-resistance. In addition, other environmental or
hereditary mechanisms might be at play, such as
combined genetic defects (e.g., protein C deficiency
combined with factor V Leiden). This combination
might result in relative risks that approximate those of
homozygosity for the factor V Leiden mutation. If
screening for factor V Leiden is done in a young woman
with an objective diagnosis of venous thrombosis, it makes
good sense to investigate also protein C, protein S, and
antithrombin deficiency. The result of screening for
generic risk factors might also have a bearing on
contraceptive decisions of the patient’s mother and sisters,
and for possible anticoagulation prophylaxis at other times
of high risk such as during immobilisation or post
partum. At first or repeat prescription of oral
contraceptives, it might pay to take a thorough personal
and family history of thrombosis and to investigate if
possible.

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