Impact of prothrombotic mutations and family history on the occurrence of intra-uterine fetal deaths

Women with fetal death were evaluated for inherited thrombophilia and family history of obstetric complications. Maternal thrombophilia was significantly associated with fetal death, while no significant increase of recurrences was recorded. Moreover, family history of obstetric complications was significantly associated with the occurrence of fetal death.

haematologica 2002; 87:1118-1119
(*http://www.haematologica.org/2002_10/1118.htm*)

The role of inherited thrombophilias in the occurrence of fetal loss has been established.[1-4] We do not know whether the risk of recurrence of late fetal loss is higher in women carrying inherited thrombophilia. Women (n=84) with at least one previous intra-uterine fetal death (IUFD)[5] who were enrolled between February 1998 and July 2001 in three Atherosclerosis and Thrombosis Centers, were screened for acquired and inherited thrombophilias.

A comprehensive work up for recurrent fetal loss was performed in all cases. Unexplained fetal loss was defined as previously described.[1] As controls, 108 healthy parous women with uneventful pregnancies were enrolled. All were Caucasian women from Southern Italy. Information about personal and family history (first-degree relatives) of IUFD, pre-eclampsia (PE), and fetal growth restriction (FGR) was collected by interviews and confirmed by means of the patients' records.

The study was carried out according to the principles of the Declaration of Helsinki. Blood samples were collected in 3.8% trisodium citrate and treated as previously described.[1]

Antiphospholipid antibodies-lupus anticoagulant (LA) and IgG anticardiolipin antibodies (aCL), antithrombin, protein C, amidolytic and immunologic and total and free protein S antigen were determined in all patients, as reported.[6] Leukocyte DNA was obtained from frozen blood and factor V Leiden and factor IIA^{20210} mutations investigated, as described.[6]

All the analyses were performed according to the Statistical Package for Social Science (SPSS 6.1 for Macintosh). The significance of differences in means was evaluated by non-parametric tests, whereas the significance of any difference in proportions was tested by χ^2 statistics. A multivariate regression analysis was performed to correct the associations found for potential confounding variables such as age, gravidity, FV Leiden, FII A^{20210} allele and a family history of obstetric complications.

Table 1 shows the features of all cases and controls. Twenty-two (26.2%) women suffered from an IUFD in the second trimester (i.e. ≤ 24 weeks), while 63 did so in the third trimester. Ten (11.9%) cases showed the FV Leiden (all heterozygous), while 11 (13.1%, 1 with both the mutations) were heterozygous for the FII A^{20210} allele.

Six (7.1%) women had a confirmed presence of antiphospholipid antibodies. Of them, 2 also showed the FV Leiden mutation and 1 both the mutations. Among controls, 2 (1.9%) carried the FV Leiden (OR: 7.9; 95% CI: 1.7-36.8) and 5 the FII A^{20210} allele (4.6%, OR: 3.1; 95% CI: 1.0-9.2). Multivariate analysis (Table 2), correcting for the potential confounding variables, age, gravidity, FV Leiden or FII A^{20210} allele, and family history, showed that prothrombotic mutations were independently associated with IUFD (OR 6.3; 95%CI: 1.0-41.1 and OR: 4.2; 95%CI: 1.0-17.1 for FV Leiden and FII A^{20210}, respectively). Moreover, a family history of obstetric complications was also independently associated with the occurrence of a first IUFD (OR: 8.4; 95%CI: 1.5-46.4). Four cases suffered from venous thrombosis (of them, two with the FV Leiden and one with both the prothrombotic mutations).

Table 1. Clinical features and coagulation screening of cases and controls.

	Patients (n=84)	*Controls (n=108)*
Age (median and range)	32.5 yr. (22-44)	31 yr. (range 19-40)
Parity (median and range)	0 (0-3)	2 (1-5)
Number of early fetal losses (median and range)	1 (0-3)	–
N. of patients with early fetal loss	42 (50%)	–
FV Leiden mutation^	10 (12.0%)	2 (1.9%)*
FII A^{20210} mutation^	11(13.1%)	5 (4. 6%)°
aPL	6 (7.1%)	1(0.9%)
Other	2 protein S deficiency§	0

**p= 0.002; °p= 0.04; §1 with recurrence. ^The frequencies observed were not significantly different from those found in a general population from the same ethnic background.[7]*

Table 2. Estimated risks of a first event and of recurrence. Logistic regression.

	First IUFD OR (95%CI)	*Recurrence^ OR (95%CI)*
Factor V Leiden	6.3 (1.0-41.1)	1.3 (0.2-6.7)
Factor II A^{20210}	4.2 (1.0-17.1)	0.5 (0.1-3.9)
Family history	8.4 (1.5-46.4)	4.5 (0.9-22.9)

^In this group 1 woman (7.1%) had the FII A^{20210} allele, 2 (14.3%) FV Leiden mutation and 1 protein S deficiency. Variables entered in the model: age, gravidity, FV Leiden, FII A^{20210} allele, family history.

Fourteen (16.7%) cases had a recurrent IUFD.

Four out of these 14 (28.6%) women with recurrence showed an inherited thrombophilia. When we compared thrombophilic women with one IUFD to thrombophilic women with recurrence, a not significant difference of inherited risk factors was recorded. No woman with more than one risk factor had a recurrence of IUFD. Family history of obstetric complications (1 IUFD, 4 PE, 2 *sine causa* FGR) was registered in 7 (8.3%) cases : one of them carried the FII A^{20210} allele and 2 the FV Leiden. In the control group, 2 (1.9%, OR: 4.8; 95%CI: 1-23.8) women showed a family history (2 IUFD) of obstetric complications: none of them carried a prothrombotic mutation. As shown in Table 2, family history showed a significant association with IUFD.

This study confirms, in a selected sample, that prothrombotic mutations are associated with IUFD.

Identification of conditions carrying an increased risk of recurrent IUFD is important for pharmacologic management of the subsequent pregnancies. At present, prospective evaluations of pregnancies in women carrying one prothrombotic mutation are lacking, and this is probably due also to the habit that physicians have of using heparin in the subsequent pregnancies of these women. Nevertheless, to know whether women with prothrom-

botic risk factors have a higher risk of recurrence is important in order to design appropriate therapeutic trials.

A family history of obstetric complications is significantly associated with IUFD.

Our data seem to indicate that there is not a higher risk of recurrence in women with a previous IUFD and an inherited thrombophilia. Moreover, this study suggests that recording the family history has an important role in choosing women who need screening for thrombophilia.

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Key words: thrombophilia, intrauterine fetal death, prothrombotic mutations.

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received June 26, 2002; accepted August 21, 2002.

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The gel enzyme technique in pretransfusion antibody screening

Some authors report that the sensitivity and specificity of the enzyme gel technique are comparable to those of enzyme tube tests,[1] while others present contrasting results.[2] However, studies using gel enzyme techniques have not been commonly performed. The aim of this study was to report our experience using a gel enzyme test as a supplementary method in a routine setting.

haematologica 2002; 87:1119-1120
(*http://www.haematologica.org/2002_10/1119.htm*)

We studied 12,789 sequential samples submitted to our laboratory for pretransfusion testing. Sera were tested by a gel indirect antiglobulin test (G-IAT) and a gel papain technique (G-PT) according to the manufacturer's procedure (Diagnostic Grifols S.A., Spain). Antibody screening was performed using a commercial panel of two untreated and papain-treated red blood cell (RBC) samples. A commercial 11-cell panel was used for antibody identification studies.

Positive results were found in 283 (2.2%) of the 12,789 samples. Irregular red blood cell antibodies were identified in 160 (1.2%) samples. Positive results were due to autoantibodies in 66 (0.5%) samples. Non-specific reactions (positive screening test followed by inconclusive results using an identification method) or false-positive results (positive screening test followed by a negative investigation) were found in 68 (0.5%) cases.

The 160 positive samples were from 91 patients and contained 192 alloantibodies: one antibody in 80 patients, two anti-

Table 1. Cumulative specificity of RBC alloantibodies.

Single antibody	*Number of patients*	*Number of samples*	*Two antibodies*	*Number of patients*	*Number of samples*
Anti-C	1*	2 (2*)	Anti-C+D	8	13
Anti-D	17	25	Anti-D+E	1	1
Anti-E	21	38	Anti-D+Le[a]	1	1
Anti-c	2	2	Anti-c+E	1	1
Anti-e	1	1	Anti-E+K	5 (1*)	12 (6*)
Anti-K	19	32	–	–	–
Anti-Jk[a]	8 (1*)	16 (2*)	–	–	–
Anti-Fy[a]	1	1	–	–	–
Anti-S	2 (1*)	2 (1*)	–	–	–
Anti-Le[a]	4	6	–	–	–
Anti-Le[b]	1	2	–	–	–
Anti-M	2	2	–	–	–
Anti-P	1	1	–	–	–

Multiple samples	*Number of antibodies*	*Number of patients*
Anti-C+D+E	2	2

**Autoantibody in association with alloantibody.*