The Role of Thrombophilia in Pregnancy

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Thromboembolism in pregnancy is a major contributor to pregnancy morbidity and mortality with potentially serious adverse effects for both mother and foetus. This article aims at exploring the impact of both inherited and acquired thrombophilia on pregnancy and to determine the appropriateness of screening for thrombophilia in pregnancy. Moreover, we focused on the principles and criteria for designing future studies and prior power analysis, i.e. determining the required number of patients in order to obtain significant and medically interpretable information.

Keywords: gene mutation, Factor V Leiden, MTHFR genes (C677T, A1298C), PAI-1 gene, Protein C, Protein S, homocysteine dosage.

Pregnancy is associated with an increased risk of venous thromboembolism (VTE) and this condition remains an important cause of maternal morbidity and mortality. Recent studies suggest that there is also a link between thrombophilia and adverse pregnancy outcomes such as foetal loss as well as VTE. Although the available data are limited and flawed, the use of anticoagulation for prevention of adverse pregnancy outcomes in women with heritable thrombophilia is increasing [1].

Approximately 50% of gestational VTE are associated with heritable thrombophilia [2]. A number of studies have examined the relationship between hereditary thrombophilia and pregnancy-related VTE. However, methodological limitations have made it difficult to obtain an accurate assessment of these risks. In a recent systematic review of 9 studies that assessed the risk of VTE in pregnant women with heritable thrombophilia, all congenital thrombophilia with the exception of homozygosis for the thermolabile methylene tetrahydrofolate reductase variant (MTHFR C677T) were found to be associated with a statistically significant increase in the risk of pregnancy-related VTE [3].

Thrombophilia can be inherited or acquired (Table 1). The most common inherited disorders during pregnancy are mutations in factor V Leiden, prothrombin gene, and methylenetetrahydrofolate reductase (MTHFR). Caucasians have a higher rate of genetic thrombophilia than other racial groups [4].

Although screening in itself cannot prevent a disease, an appropriately designed and conducted screening program is considered to be a *preventive* measure, as it aims to determine and influence risk factors, or detect and treat early stage abnormal changes that could later develop into a disease [5].

Table 1

THROMBOPHILIAS ARE INHARITED OR ACQUIRED

INHERITED
• Protein S deficiency
• Protein C deficiency
• Antithrombin III
• Factor V Leiden mutation
• MTHFR mutation
• Homozygosity to MTHFR C677T
• Homozygosity to 4G/4G mutation in PAI-1 gene
• Prothrombin G20210A mutation
• Polymorphisms in thrombomodulin gene
ACQUIRED
• Antiphospholipid antibody syndrome
• Hyperhomocysteinemia

Who should have thrombophilia screening [4]?

Thrombophilia screening is expensive and time consuming, therefore important to be targeted at the right people in due

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time. The following guidelines should identify those individuals most at risk:

- patients with a known family history of any of the inherited thrombophilia factors;
- patients with a family history of proven venous thrombo-embolism;
- patients who have developed a thrombosis with no obvious precipitating cause or at a relatively young age. Presently this is advised below the age of 50;
- women with a history of recurrent miscarriages should be screened for the lupus anticoagulant.

What medical test should be performed [4, 6-8]?

Factor V Leiden: Second-generation activated protein C resistance assay is reliable in pregnancy; if results are abnormal, evaluate for genotype for factor V Leiden mutation; if the patient is on anticoagulation therapy, consider evaluation of factor V Leiden mutation via genotype testing .

Prothrombin G20210A mutation DNA analysis.

The study we report hereby aimed at exploring the impact of thrombophilia on pregnancy and to determine the appropriateness of screening for thrombophilia in pregnancy.

Experimental part

Study population

The pilot study had a cross-sectional design; it included 30 patients referred from Bega Obstetrics and Gynecology Clinique, part of Timis County Hospital, towards the Haematology Clinique of the Municipal Hospital of Timisoara. All the assessed patients were enrolled between 2014 and 2015. The 30 patients were diagnosed with different kinds of thrombophilia prior giving birth. The diagnosis was given after consulting the gene mutation analysis for the following indicators: Factor V Leiden, MTHFR genes (C677T, A1298C), PAI-1 gene, the activity of Protein C, the activity of Protein S and homocysteine dosage.

The included patients represented approximately 1.2% of the total number of women that gave birth in that period of time.

The study patients were in their second and third trimester of pregnancy, with a gestational age between 14 and 40 weeks old, patients mean age was 30.5 years old with a minimum of 23 and a maximum of 42 years old.

Inclusion criteria for the patients were as follows:

- random thrombophilia diagnosis after the first pregnancy loss;
- at least 2 abortions of unknown cause
- the presence of mutant genes that predisposes to thrombotic events;
- age over 18 years old.

Exclusion criteria:

- abortion of other known cause;
- age under 18;
- harmful environment or living conditions which could determine abortion.

Statistical Methods

Descriptive statistics were performed as it follows: for numerical variables, Shapiro-Wilk test for normality was applied, then the mean ± standard deviation (sd) for normally distributed values, and median (InterQuartile Range) on the contrary, were provided; for category variables, the observed frequencies (percent) were provided. When comparing binary-transformed variables, Chi-square tests (either asymptotical or Monte Carlo simulation with 10000 replicates), Fisher-exact or McNemar tests were applied. For rank variables, the Wilcoxon test was applied. All reported probability values were two-tailed and a 0.05 level of significance was usually considered, while marking the very significant and marginally significant values, as well.

To investigate and describe the relationship of different medical investigations to the dichotomous variable describing abortion/live birth, unconditional logistic regression was applied [9].

A power analysis for the logistic regression was conducted and sample sizes calculated under a range of scenarios, by employing the methods proposed by Hsieh FY [10-12].

The logistic regression mode is [12]:

$$log(p/(1-p)) = \beta\_0 + \beta\_1 X$$

where $p=prob(Y=1)$, X is the predictor, and β_1 is the log odds ratio (OR). The sample size formula we used for testing if $\beta\_1=0$ or equivalently $OR=1$.

The sample size is:

$$n = (Z\_\{1-\alpha/2\} + Z\_\{power\})^2/[\, p\_1\,(1-p\_1)\,[log(OR)]^2]$$

where n is the required total sample size, OR is the odds ratio to be tested, p_1 is the event rate at the mean of the predictor X, and Z_u is the u-th percentile of the standard normal distribution.

For sample size calculation, point-estimators from literature [6, 7] were used, rather than those actually calculated based on the present study sample.

All statistical analysis was conducted with SPSS v.17 and the R software packages v.3.2.2.

Results and discussions

Results on the Study Sample

Table 2 presents the study sample with a general overall description, and table 3 shows the descriptive statistics for the two MTHFR C677T groups, i.e. positives vs. negatives. All the observed differences in clinical and laboratory investigations proved to be non-significant from statistical point of view.

An unconditional logistic regression model was attempted for the abortion outcome, but none could be fit (i.e. no statistical significance reached) with the actual data and variables.

Analysis of Statistical Power and Sample Size Calculation

Apart from the weak aspects of any cross-sectional study and the shortcomings in the present study design itself, a lack of statistical power was suspected to be the real, main reason behind the total failure in finding any association between the pregnancy outcome and either the presence of thrombophilia-specific genetic mutations or the haematological determinations.

Therefore, an analysis of the required sample size for the logistic regression was conducted, considering $alpha=0.05$ for the significance level and $1\text{-}beta=0.8$ as level of statistical power. Factor V Leiden as the main predictor for the abortion/live birth was taken, with an estimated OR coefficient of 1.67 and an event rate of 3.7% [7], which resulted in a minimum sample size of 80, for a one-variable logistic regression model.

Subsequently, we considered multi-variable models, varying the OR values and the event rates, each scenario with small, medium and large squared correlation coefficients of 0.1, 0.3, and 0.5, respectively.

Tables 4-6 show the required sample size for the main event rate in a [0.01-0.08] range, and OR over the [0.1-0.9] range. Figure 1 illustrates the relationships between the event rate and the R-squared, for OR=0.6 and OR=0.1, respectively.

As shown in table 1, the study sample was small, compared to the size required for reaching statistical significance and reasonable power, as calculated values in tables 4-6 demonstrate. Moreover, when comparing these actual data descriptive statistics to the *normal* values recommended by accredited laboratories, they seem to indicate the study subjects as being healthy and free of any particular obstetrical risk. A possible explanation of these good laboratory results might be the ongoing treatment of the patients: they all received preventive medication with low molecular weight heparin, as recommended [4].

Total	30 patients	27 patients with data regarding Factor V G1691A-Leiden, MTHFR C677T, MTHFR A1298C, Factor II G20210A	
Age	30.5 ± 4.95	median = 30 IQR (26;33)	(min – max) → (23 – 42)
Age over 35 years	3 patients		
Factor V G1691A-Leiden	1 patient had MTHFR C677T (heterozygote mutation) + MTHFR A1298C (homozygote mutation) Factor II G20210A absent		
MTHFR C677T	7 absent	3 homozygote	17 heterozygote
MTHFR A1298C	14 absent	1 homozygote	12 heterozygote
Factor II G20210A	25 absent	2 homozygote	

Table 2
SAMPLE DESCRIPTION

	MTHFR C677T positive (homo + hetero-zygote)		**MTHFR C677T negative**	
	N total		**N** total	
Patient age [a]	20	30.50 (26 ; 34)	7	30 (26 ; 32)
Gesta[a]	20	2 (1 ; 3)	7	2 (2 ; 2)
Para[a]	20	1 (0 ; 1)	7	1 (1 ; 1)
Early pregnancy losses before diagnosis[a]	20	1 (1 ; 2)	7	1 (1 ; 1)
After thrombophilia diagnosis (current pregnancy)				
Live birth[b]	20	17 (85%)	7	5 (71.4%)
Live birth gestational age[a]	20	34 (34 ; 38)	5	33 (33 ; 33)
Abort (GA < 24 weeks) [b]	17	3 (15%)	7	2 (28.5%)
Abort gestational age [a]	3	14 (13 ; 15)	2	15 (13 ; 19)
Foetus gender (M) [b]	18	13 (72.2%)	6	5 (83.3%)
Foetus weight [g] [a]	17	2570 (2150 ; 3400)	5	2510 (2300 ; 2560)
Foetus length [cm] [a]	17	44 (36 ; 48)	5	37 (37 ; 40)
Apgar index[a]	17	8 (8; 9)	5	7 (7 ; 8)
D-DIMER[c]	11	0.35 ± 0.19	7	0.37 ± 0.23
AN fxa[c]	4	0.56 ± 0.18	3	0.34 ± 0.04
IgG [GPLU/mL] [c]	8	2.35 ±0.42	6	2.8 ± 2.07
IgM [MPLU/mL] [c]	7	1.21 ± 0.61	6	1.52 ± 0.59

Table 3
DESCRIPTIVE STATISTICS FOR THE TWO GROUPS OF MTHFR C677T MUTATION

continuated table 3

Lupic anticoagulant [sec] [c]	11	33.03 ± 6.34	5	42.96 ± 4.22
Antithrombin III [%][c]	16	84.84 ± 35.53	5	84.23 ± 47.17
Protein C [%][c]	14	104.3 ± 13.3	5	101.04 ± 18.78
Protein S [%][c]	14	79.81 ± 20.53	5	97.32 ± 18.54
Homocystein [umol/l] [c]	11	7.728 ± 4.486	6	8.395 ± 2.162
[a] median (IQR); [b] n (%); [c] m ± s				

Table 4

REQUIRED SAMPLE SIZES WHEN R-SQUARED = 0.1

OR	Event rate / R-squared = 0.01							
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08
0.1	167	84	57	43	36	30	26	23
0.2	341	172	117	88	71	60	52	47
0.3	608	308	208	158	127	108	93	82
0.4	1050	530	358	271	219	184	160	142
0.5	1834	927	624	473	382	322	279	247
0.6	3377	1706	1149	871	704	593	514	454
0.7	6924	3498	2357	1786	1443	1216	1053	932
0.8	17692	8937	6019	4561	3688	3106	2691	2380
0.9	79356	40083	26998	20459	16540	13930	12069	10674

Table 5

REQUIRED SAMPLE SIZES WHEN R-SQUARED = 0.3

OR	Event rate / R-squared = 0.03							
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08
0.1	214	109	73	56	46	39	33	30
0.2	439	221	150	113	91	77	67	60
0.3	781	396	267	203	163	139	120	106
0.4	1350	681	460	349	281	237	206	183
0.5	2359	1191	803	609	491	414	359	317
0.6	4341	2193	1477	1120	906	763	661	584
0.7	8903	4497	3030	2296	1856	1563	1354	1199
0.8	22747	11490	7739	5864	4741	3993	3460	3060
0.9	102029	51536	34711	26304	21266	17910	15517	13724

Table 6

REQUIRED SAMPLE SIZES WHEN R-SQUARED = 0.5

OR	Event rate / R-squared = 0.05							
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08
0.1	300	152	102	78	64	54	46	42
0.2	614	310	210	158	128	108	94	84
0.3	1094	554	374	284	228	194	168	148
0.4	1890	954	644	488	394	332	288	256
0.5	3302	1668	1124	852	688	580	502	444

continuated table 6

0.6	6078	3070	2068	1568	1268	1068	926	818
0.7	12464	6296	4242	3214	2598	2188	1896	1678
0.8	31846	16086	10834	8210	6638	5590	4844	4284
0.9	142840	72150	48596	36826	29772	25074	21724	19214

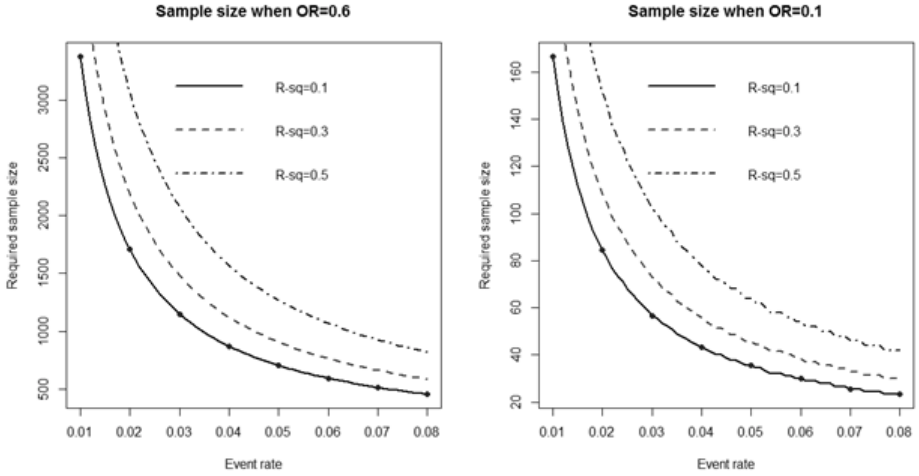

Fig. 1. Required sample size in order to reach statistical significance alpha=0.05 and power=0.8, when the event rate ranges between 1% and 8% and OR is 0.6 or 0.1. Distinct curves were drawn for three R-squared values. Factor V Leiden was taken as the first predictor in the logistic model, with point estimates from Carp H et al. [7]

Taking all the descriptives into consideration, the failure in finding any significant regressional model to fit the relationship between different medical investigations and the final obstetrical outcome (i.e. whether or not an abortion happened) was not surprising. The only exception was an isolated significant contribution of the protein S values, which we interpreted as being due to chance or random error, with little connection to other literature reports or plausible medical explanation in the context of no other interaction.

The supposition of lack of statistical power was fully confirmed by the results of the systematic analysis, the required sample size being orders of magnitude larger than the actual study sample. For example, for values of OR~0.5, event rate of ~0.08 in case group as in Lund M et al. [6], with a medium correlation between the model predictors (R-squared=0.3), the required number of subjects in the case group is 317, as shown in table 5, i.e. more than ten-fold the sample size in the present study.

Conclusions

Overall, good planning, sensible inclusion/exclusion criteria, and careful collection of medical data should be considered before any screening for thrombophilia in general, and among women at reproductive age, in particular. On the other hand, a systematic screening and clear recommendations for the family doctors would certainly help raising the awareness of this medical problem and its entailing obstetrical risks.

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