

Thrombophilia Work-up in Females with Venous Thromboembolism in Association with Oral Contraceptive Use: Results, Strategy and Clinical Application of Testing

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Abstract

Background: Oral contraceptive use is one of the most common causes of venous thromboembolism in females in a reproductive age. The usefulness of thrombophilia work-up in this scenario has been discussed in the last years especially in the term of financial cost and clinical implication. In the case of testing, it is necessary to keep appropriate timing and conditions for every assay.

Methods: We analyzed thrombophilia work-up in a large cohort of 700 females with venous thrombosis in association with combined contraceptive pills use. We focused on laboratory thrombophilia work-up in the term of an appropriate strategy of testing, the influence of oral contraceptives use, presence of acute thrombosis and type of anticoagulation therapy.

Results: We found high frequency of inherited thrombophilia and antiphospholipid syndrome in our study group (45%). F V Leiden was absolutely the most frequent (30%). The frequency of inherited thrombophilia and antiphospholipid syndrome in the subgroup of females with spontaneous VTE reached significant statistically difference ($p < 0.0001$) in comparison with the subgroup with VTE in risk situation. Based on current approach to testing, we assume the result had clinical impact only in 10%-11% of cases. So that nowadays we prefer personalized and individual approach to testing.

Conclusion: Thrombophilia work-up involves many tests based on different principles so that the perfect knowledge, what, when and how is inevitable for correct results and clinical interpretation. In spite of a high frequency of thrombophilia, the testing is recommended selectively only when the result has clinical consequence.

Summary: Oral contraceptive use is one of the most common causes of venous thromboembolism in females in a reproductive age. We analyzed thrombophilia work-up in a large cohort of 700 females with thrombosis in association with combined contraceptive pills use. In this article, we focused on laboratory thrombophilia work-up in the term of an appropriate strategy of testing and the discussion of usefulness of this testing generally. Thrombophilia work-up involves many tests based on different principles. So that the perfect knowledge, what, when and how is inevitable for correct results and clinical interpretation. We recommend testing only in cases, when the result has impact on clinical management.

Keywords: Venous thromboembolism; Oral contraceptive use; Thrombophilia work-up

Abbreviations APTT: Activated Partial Thromboplastin Time; AT: Antitrombin; APS: Antiphospholipid Syndrome; COC's: Combined Oral Contraceptive Use; dRVVT: diluted Russell's Viper Venom Time; DOACs: Direct Oral Anticoagulants; LA: Lupus Anticoagulant; LMWH: Low Molecular Weight Heparin; PC: Protein C; PS: Protein S; PT: Prothrombin Time; VKA: Vitamin K Antagonist; UFH: Unfractionated Heparin; VTE: Venous Thromboembolism

Introduction

Venous thromboembolism (VTE) is multifactorial disease with incidence 1/1000 [1]. Combined oral contraceptive use (COC's) and pregnancy are common cause of VTE among women in a productive age. "Low dose" COC (<50 µg ethinylestradiol) are associated with a three to six-fold increased relative risk of VTE (World Health Organization, 1995) but an absolute risk remains low (three to four VTE per 10,000 women per year using OC [2,3]). The third generation of COC is slightly more thrombogenic than the second one [4]. A variety of acquired and inherited risk factors interact with COC's to increase risk of VTE. Regarding inherited thrombophilias, prevalence of FII 2010A is 3% and F V Leiden 2% in Czech population [5]. Frequency of other inherited thrombophilia such as antitrombin (AT)

deficiency, protein C (PC) and protein S (PS) is not precisely known in our country almost 0.5 million of Czech women are taking COC. Thrombophilia work-up is not routinely recommended before prescription of COC [6,7].

In spite of all these recommendations, it is still done in our country. However, there is no consensus about thrombophilia work-up even in female with VTE in association with COC's. Current approach is to make thrombophilia work-up selectively because of a lack clinical utility. If it is thrombophilia work-up done, it is necessary to keep appropriate strategy, what, when and how to make it. Many errors are made in daily routine clinical praxis and it results in spurious conclusions, false diagnosis and the recommendations. The psychological consequences are also not negligible.

Aim of the Study

We analyzed thrombophilia work-up in cohort of 700 females with VTE in association with COCs in period 1997-2016. All thrombotic events DVT, PE, thrombosis at unusual site were objectively documented. This study started in 1997 when thrombophilia work-up was routinely done in this setting.

We focused on two main points:

- An appropriate time of thrombophilia work-up (what, when and how) to avoid the potential errors. We analyzed particular type of thrombophilia with regard to the principle of used test (PCR, coagulation, chromogenic etc.). The influence of COC, acute thrombosis and type of anticoagulation therapy were always carefully assessed.
- The utility of thrombophilia work-up in this setting. It is still largely done in our country; some physicians even make the testing before COC prescription. As time has been going on the usefulness of testing has faded mainly due to the lack of clinical consequences.

Materials and Methods

For that purpose we made thrombophilia work-up in cohort of 700 women with VTE in association with COCs. All of our investigated women took the COC but the precise type is not available in all females. The laboratory testing was done either after withdrawal of anticoagulation therapy or on anticoagulation therapy.

What we tested?

We made following tests: F V Leiden mutation, F II20210A mutation, antithrombin, protein C, protein S, homocysteine level, presence of lupus anticoagulant (LA), anticardiolipin level and anti beta2 glycoprotein I antibody.

How have we made it?

Blood samples were collected by venipuncture into plastic tubes containing either 1/10 volume of 3.8% sodium citrate for coagulation assays or 1/10 volume of 0.5 mol/L sodium ethylenediamine tetraacetic acid for DNA extraction after centrifugation (15 min at 2500 g) for prothrombin time (PT), activated partial thromboplastin time (aPTT) and AT assays or after double centrifugation (10 min at 1500 g) for protein C, protein S and LA assays. Protein C was determined by coagulation assay using Staclot Protein C (STAGO D, Asnieres, France; normal value 70%-130%).

A Staclot Protein S (STAGO; normal value 65%-140%) kit was used for determination of protein S. Antithrombin was determined by chromogenic assay used the Stachrom AT kit (STAGO D; normal value 80%-120%). The normal range for protein C, protein S and AT was obtained by examination of 100 healthy individuals (50 men, 50 women) from our region and normal values were compared with the normal range recommended by the manufactures. The diagnosis of a PC, PS or AT deficiency should only be accepted after multiple testing. The diagnosis of antiphospholipid syndrome (APS) was made by these tests. To detect LA, the following screening assays were performed:

- aPTT, PTT Automate (STAGO D)
- aPTT with high sensitivity to LA, PTT-LA (STAGO D)
- Tissue thromboplastin inhibition test
- Diluted Russell's Viper Venom Time [dRVVT]

If one of these tests was positive the second determination was performed after 6 to 8 weeks and the test with hexagonal phospholipids (Staclot LA, STAGO D) was carried out for confirmation. A solid-phase immunoassay technique was used to quantify ACA levels and anti beta2 glycoprotein I antibody. Only moderate or high elevations were considered as positive results and also in the case of positivity from the first sample; the second evaluation was carried out after 6 to 8 weeks.

Both antibodies tested against the International Standard in IgM and IgG classes. The polymerase chain method was used for FV Leiden and FII G20210A determination. Genomic DNA was isolated from peripheral blood leukocytes using high pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). F V Leiden and prothrombin G20210A mutations were determined by the LightCycler 1.5 commercial Kit (Roche Diagnostics, Mannheim, Germany).

Level of homocysteine was determined immunochemically in an analyzer (DPC Immulite 2000). Normal level for homocysteine (normal range 12.5-17 mmol/L) was obtained by examining 100 healthy individuals (50 men, 50 women after strictly 12 hours of fasting and patient could not take folic acid, vitamin B6 or B12. We strictly kept following conditions of measurements: F V Leiden and F II20210a mutation: Genetic testing can be performed at any time, regardless of acute or chronic VTE, therapy. We usually tested these mutations during anticoagulation therapy.

- **Protein C:** Protein C clotting assay could be influenced by APC resistance, ↑ F VIII and LA. The testing was not allowed to make on therapy with vitamin K antagonist (VKA) in liver disease and acute VTE [8]. We measured protein C always after cessation of anticoagulation therapy.
- **Protein S:** Protein S clotting assay, PS activity is ↓ in acute VTE, in inflammatory states on therapy with vitamin K antagonist (VKA) in liver disease, protein-losing enteropathy and what is important on COCs and two months after cessation COC [9,10]. We measured protein S always after cessation of anticoagulation therapy.
- **Antithrombin:** Decreased activity we can find in acute thrombosis, heparin therapy and slightly on COC's. On the contrary, level can be slightly increased on coumarine therapy. Antithrombin was tested usually twice immediately after diagnosis in case of severe thrombosis as proximal DVT, PE or thrombosis at unusual site with the idea to rule out suspicious AT deficiency. Routinely was done it after cessation of anticoagulant therapy. Data in the literature on the effect of estrogens on PC and AT levels are

inconsistent either no effect has been reported or a slight ↓ in AT and mild ↑ in protein C [11,12].

- **LA:** It is not recommended to make it in acute thrombosis and on all kinds of anticoagulation therapy. The best time is at least one month after cessation of coumarine or one week after DOACs therapy. ACA and anti beta2 glycoprotein I antibody: It can be done whenever is necessary (Table 1).

Thrombophilia	Acute VTE	COCs	Coumarine	Heparin (UFH + LMWH)
F V Leiden mutation	Y	Y	Y	Y
F II20210a mutation	Y	Y	Y	Y
Antithrombin	P ↓	P ↓	P ↑	P ↓
Protein C	P ↑	P	N	Y
Protein S	P ↓	N	N	Y
LA	N	N	N	N
ACA+anti beta2 Gl 1	Y	Y	Y	Y
Homocystein	Y	Y	Y	Y

Table 1: Influence on thrombophilia test results. Y- it's possible to evaluate, N-it's not possible to evaluate, P-possible, but can be slightly ↓ or ↑, UFH-unfractionated heparin, LMWH-low molecular weight heparin.

Since October 2012 it has been possible to treat VTE with rivaroxaban (Xarelto) and later on with other direct oral anticoagulants (DOACs). So we had to keep the conditions for thrombophilia work up. DOACs function as inhibitors of FXa or FIIa and therefore they have impact on clot-based and chromogenic assays that rely on either of these factors [13].

DOACs can lead to prolongation of aPTT or PT [14]. Regarding inherited and acquired thrombophilia screening, DOACs may cause false-positive lupus anticoagulant test results both with APTT-based and with dRVVT based screening and confirmatory assays. Clot-based protein C, protein S and activated protein C resistance assays may lead to overestimation in the presence of DOACs it means we can yield falsely normal results.

However, we recommend these tests after cessation of DOAC therapy, because of potential underlying deficiencies can be masked [15]. Clinical data are shown in following tables but the analysis is largely discussed elsewhere [16].

The characteristics of our cohort are shown in Table 2.

Parameters	
Number of female	700
Mean age at the time of thrombophilia work-up	32 years, age range (17-55)
Mean age at the time of the first VTE	27 years, age range (17-51)
The average duration of OC use	46 months

Table 2: Characteristics of our cohort.

The characteristics of our cohort are shown in Table 3.

Type of thrombophilia	Number	Percentage (%)
F V Leiden mutation-overall	210	30.00%
heterozygous trait	195	
homozygous trait	15	
FII 20210A	34	4.9
APS	24	3.4
Protein C deficiency	6	0.9
Antithrombin deficiency	13	1.9
Protein S deficiency	6	0.9
FVL+APS	10	1.4
Other combinations	10	1.4

Table 3: Frequency of respective types of thrombophilia.

The prevalence of F V Leiden mutation is highly dominant inherited thrombophilia. On the other hand, hyperhomocysteinemia has not been found at all. We have also found in 20 cases combined thrombophilia (mostly in 10 female) F V Leiden+APS. The results are important from clinical point of view. The frequency of inherited thrombophilia (+APS) in spontaneous VTE and VTE in risk situation are summarized in Table 4.

Thrombophilia	Number	Percentage (%)
Overall thrombophilia	313	45
Thrombophilia in spontaneous VTE	217	31
Thrombophilia in VTE in risk situations	96	14

Table 4: The frequency of inherited thrombophilia (+APS) in spontaneous thrombosis and risk situations.

The frequency of inherited thrombophilia and APS in the subgroup of female with spontaneous VTE reached significant statistical difference ($p < 0.0001$) in comparison with the subgroup with VTE in risk situation.

The results were statistically assessed using Fishers exact test, programme NCSS 2004).

Discussion

Thrombophilic defects were identified in about 50% of women presenting with an oestrogen related VTE. We found also high frequency of inherited thrombophilia and APS in our study group (45%). If we compare the individual types of thrombophilia we found similar percentage of F V Leiden (30%) as in other previous studies (20%-35%) [11,12,17]. Concerning F II20210A we identified heterozygous form less frequently (5%) in comparison with other studies (14%) [6,13,14]. Deficiencies of AT, protein C and S were diagnosed in 1%-2%. It is in concordance with other studies (1%-4%) [14,18]. However, we would like to discuss the utility of testing in this scenario.

A big boom of thrombophilia work-up was at the time of beginning of our analysis. It was a period of a lot of new information about particular tests and their limitations. These years all females with VTE in association with COC's were tested for thrombophilia. A lot of doctors still recommend thrombophilia work-up even routinely before COC prescription in our country. This tendency was further accelerated with reduced cost of testing. However, the utility of thrombophilia work up faded as time has been going on. Many articles have not shown a benefit of testing in the term of the reduction of recurrent (VTE) [19,20]. However, adherence to rational guidelines is still very variable in Europe [21].

We agreed and kept 4 P proposal of thrombophilia work-up [7]. It consists of appropriate selection which patient to test, pre-test counseling, correct interpretation the test results and education and advice to the patient. Nowadays we recommend the thrombophilia testing individually and selectively particularly in women of childbearing age. The crucial point is clinical benefit for female.

Concerning our cohort, the testing did not have the consequences for the duration of anticoagulation therapy in most of the cases [22,23]. However, at least 10%-11% of females from our cohort were candidates for prolonged anticoagulation therapy (female with F V Leiden mutation in homozygous form, female with antithrombin, protein C and S deficiency, APS, combined thrombophilia) and also this finding can have the impact on management of pregnancy and puerperium in the future.

10-11% of females from our cohort were candidates for prolonged anticoagulation therapy (female with F V Leiden mutation in homozygous form, female with antithrombin, protein C and S deficiency, APS, combined thrombophilia) and also this finding can have the impact on management of pregnancy and puerperium in the future.

If we calculate costs of testing based on the rules mentioned above it is non-profit work and also for some female positive result is stressful. On the other hand, a lot of females still ask for testing in our clinical practice based on the campaign in newspapers and periodicals. It is more advisable to recommend thromboprophylaxis with LMWH in risk situations for VTE [24]. We continue on testing only in these scenarios: Serious VTE (as iliofemoral thrombosis, pulmonary embolism-intermediate or high risk) without any transient risk factor, thrombosis at unusual site and recurrent superficial thrombophlebitis without varices.

Conclusion

Almost half a million of Czech women are taking oral contraceptives. COC's is frequent risk factor for VTE in females in reproductive age. However, the absolute risk of thromboembolism is very low in healthy women and the potential risk of VTE with the use of COCs is far less than the risks associated with unintended pregnancy. We are persuaded that personalized and individual approach is better than unselected testing. To get correct results clinician has to be aware of all pitfalls of laboratory evaluation. Therefore, we prefer to do thrombophilia work up in the centers for thrombosis and hemostasis.

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