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Relation Between Preclinical Atherosclerosis and Venous Thromboembolism in Patients With Thrombophilias – Longitudinal Study

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Summary

Preclinical atherosclerosis may represent a risk factor for venous thromboembolism (VTE). In longitudinal study we followed longitudinally 96 patients (32 men) with thrombophilias with (n=51) and without (n=45) history of VTE. In both groups we studied the changes of preclinical atherosclerosis at peripherally located arteries detected by ultrasound. In addition, we assessed changes in selected risk factors of atherosclerosis. During the mean follow-up of 56.0±7.62 months we did not find significant change in preclinical atherosclerosis defined as Belcaro score in either group (–3 % in the VTE group vs 0 % in non VTE group). Significant increase in body mass index (1.03±1.98 kg*m^{-2}, resp. 1.21±1.67 kg*m^{-2}, $p<0.01$) and non-significant increase in systolic blood pressure were detected in both groups. Waist circumference increased significantly only in patients without VTE (4.11±7.84 cm, $p<0.05$). No differences in changes of risk factors under study between both groups were detected. In summary, patients with thrombophilia and history of VTE showed no evidence of greater progression of atherosclerosis or increase in traditional risk factors of atherosclerosis than patients with thrombophilia without history of VTE. Unfavorable changes of body mass index, waist circumference and systolic blood pressure were detected in both groups during study period.

Key words

Preclinical atherosclerosis • Ultrasonography • Venous thromboembolism • Thrombophilia

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Introduction

The causative relation between atherosclerosis and venous thromboembolism (VTE) remains controversial although both atherosclerosis and VTE may share common risk factors and pathophysiological features including age, obesity, increased waist circumference, metabolic syndrome (Ageno *et al.* 2006, 2008) and endothelial dysfunction (Migliacci *et al.* 2007). In a prospective case – control study of 299 unselected patients with deep vein thrombosis (DVT), more carotid plaques had been found in patients with unprovoked DVT compared to patients with DVT of known origin (Prandoni *et al.* 2003). In accordance with this finding, in a retrospective case – control study, more coronary calfications were found in patients with unprovoked VTE compared to controls without VTE (Hong *et al.* 2005). In other studies, more cardiovascular events have been found in patients after VTE (Becattini *et al.* 2005, Bova *et al.* 2006, Sørensen *et al.* 2007). In contradiction with these findings, results of two population – based studies did not reveal that atherosclerosis is predictive of VTE (van der Hagen *et al.* 2006, Reich *et al.* 2006).

In our previous cross-sectional study (Auzký *et*

al. 2010) we studied whether in patients with thrombophilias, who are at particular risk of VTE, the presence of preclinical atherosclerosis and traditional cardiovascular risk factors are associated with VTE. In this study we did not find strong association between preclinical atherosclerosis and VTE. Nevertheless, we found obesity and hypertension to be strongly associated with a history of VTE in patients with thrombophilias indicating that these factors might be of importance in this population. Therefore, in recent longitudinal study we focused on changes of preclinical atherosclerosis and selected cardiovascular risk factors in the same population in prospective manner. The particular aim of this study was to detect differences between patients with VTE and without VTE in changes of subclinical atherosclerosis, body weight, fat distribution and systolic blood pressure in approximately 5 year period.

Methods

Study group consisted of patients with already known thrombophilias who participated in the previous study (Auzký *et al.* 2010). The ethics committee of the Institute for Clinical and Experimental Medicine and Thomayer´s hospital, Prague, approved the study and all participants signed an informed consent. All participants were interviewed about their medical history according to a standardized protocol. Medical history was focused on the type of thrombophilias confirmed by a hematologist.

For hematological analyses DNA was isolated from whole blood by using the column DNA isolation method (Macherey-Nagel GmbH & Co.KG, Germany). The presence of thrombophilic gene mutations was evaluated by real-time PCR method by using kits designed for the detection of the G20210A mutation in the gene for prothrombin, the C677T mutation in the gene for methylentetrahydropholate reductase (MTHFR), the A1298C mutation in the gene for MTHFR and the G1691A variation detection in the human factor V gene (Leiden variation) (Institute of Applied Biotechnologies a.s., Czech Republic).

In addition, information about history of myocardial infarction, angina pectoris, transient ischemic attack, stroke, intermittent claudication, arterial revascularization, tobacco smoking, diabetes mellitus, hypertension, dyslipidemia, cancer and current medication including hormonal therapy was obtained. Body height, weight, waist circumference and blood pressure were also measured according to a standardized protocol. Body mass index was calculated as weight in kg over squared height in meters.

For statistical analyses, patients with a history of regular smoking were defined as smokers, hypertension was defined as the use of antihypertensive drugs and/or systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, diabetes mellitus was defined as self-reported regular use of diabetic diet and/or anti-diabetic drugs, and dyslipidemia was defined as self-reported use of lipid-lowering drugs or knowledge of higher blood lipids, also reported by the patients. Data regarding a history of VTE, location of venous thrombosis, the cause of the VTE and use of anticoagulant treatment were obtained from the medical records of each subject.

All subjects underwent bilateral ultrasound assessment of the carotid and femoral arteries by Toshiba APLIO 50 XV (Tochigi, Japan) ultrasound system with a 7.5-10 MHz linear array transducer. The carotid arteries were examined with the patient in the supine position and the neck rotated 45 degrees in the direction opposite the site being examined. The images of the common, internal and external carotid arteries were obtained. Subsequently, the femoral arteries were examined with the patients in supine position. The images of the common femoral arteries and their bifurcations were obtained. All arteries were scanned by transverse and longitudinal projection. The images were subsequently read by the sonographer blinded with regard to presence/absence of history of VTE and concomitant use of medication including anticoagulants.

The presence of preclinical atherosclerosis was defined by semiquantitative classification – the Belcaro score. The classification was based on measurements obtained in common carotid arteries and their bifurcations and in common femoral arteries and their bifurcations. The classification by Belcaro has been described elsewhere (Belcaro *et al.* 1996). This classification evaluates the degree of preclinical atherosclerosis based on ultrasound criteria as follows: Class I: normal – three ultrasonic layers (intima-media, adventitia, and periadventitia) clearly separated, no disruption of lumen-intima interface for at least 3.0 cm, and/or initial alterations (lumen-intima interface disruption at intervals of <0.5 cm). Class II: intima-media granulation, granular echogenicity of deep, normally unechoic intimal-medial layer and/or increased intima-media thickness (>1 mm). Class III: plaque without hemodynamic disturbance, localized wall thickening and increased density involving

Table 1. Prevalence of thrombophilias within the study population.

	History of VTE n = 51*	**No history of VTE n = 45****	**p (χ^2 test)**
Factor V Leiden heterozygotes	27 (52.9)	29 (64.4)	0.302
Factor V Leiden homozygotes	4 (7.8)	2 (4.4)	0.681
Prothrombin G20210A heterozygotes	6 (11.8)	14 (31.1)	0.025
Protein C, S deficiency	1 (2.0)	1 (2.2)	1.00
Antithrombin deficiency	2 (3.9)	0	0.497
Lupus anticoagulans antibodies	2 (3.9)	0	0.497
*Isolated MTHFR mutation****	11 (21.6)	5 (11.1)	0.280

Data are expressed as n (%). *One patient was carrier of both factor V Leiden heterozygous mutation and prothrombin G20210A heterozygous mutation and one patient was carrier of both factor V Leiden heterozygous mutation and protein C deficiency – these patients appear as two additional entities. ** Five patients were carriers of both factor V Leiden heterozygous mutation and prothrombin G20210A heterozygous mutation and one patient was carrier of both prothrombin G20210A heterozygous mutation and protein S deficiency – these patients appear as additional five entities. *** Data comprises all subjects with isolated MTHFR muration (C677T, A1298C; heterozygous or homozygous). MTHFR = methylenetetrahydrofolate reductase; VTE = venous thromboembolism.

all ultrasonic layers, intima-media thickness >2 mm. Class IV: stenotic plaque, as in 3, but with hemodynamic stenosis on duplex scanning (sample volume in the center of the lumen), indicating stenosis >50 %. The highest value of the Belcaro score found in the arterial system (either in carotid or in femoral arteries) in each subject was used for further analyses (Belcaro score combined). In addition, we analyzed separately Belcaro score for carotid (Belcaro score carotids) and femoral arteries (Belcaro score femorals); similarly in this case we used the highest value for subsequent analysis.

The data are presented as percentages for categorical variables and means for continuous ones. Between groups comparison of continuous variables was calculated using unpaired t-test; for discrete variables, a chi square test (Fischer exact test) was applied. Analyses of changes of selected factors were performed by paired t-test; differences in these changes were performed by unpaired t-test. All analyzes were performed by software STATA.

Results

Complete data were obtained from 96 patients (32 men). The average duration of follow-up was 55.80± 8.03 month in the VTE group and 56.46±7.17 months in the group of patients without a history of VTE. Twenty seven patients (21.6 %) from original study population were lost to follow-up. Two women died during the follow-up, both were from VTE group. No patient in the study reported recurrent VTE during follow-up. Thirty patients (59 %) from the VTE group were on warfarin at the time of analysis whereas no patient from non-VTE group was on anticoagulant therapy.

Table 1 shows distribution of particular thrombophilias and their combinations in both groups under study. The most frequent thrombophilia was Factor V Leiden heterozygous mutation, which was present in 56 patients. Representation of prothrombin heterozygous mutation was significantly higher in patients without history of VTE.

Table 2 shows data of patients at the end of follow-up. No significant differences were found in any cardiovascular and other factors under study including values of all three types of Belcaro score; the latter remained non-significantly higher in patients with history of VTE as in previous cross-sectional study.

Table 3 shows changes of main factors studied in previous cross-sectional study (preclinical atherosclerosis, body mass index, waist circumference and systolic blood pressure) in patients with and without VTE. We did not observe any significant changes in the case of atherosclerotic changes defined as Belcaro score combined (evaluating changes in carotid and femoral arteries together), similar results were found separately for Belcaro score in carotid and femoral arteries (not shown). No significant changes were found also for systolic blood pressure. In contrast, body mass index increased significantly in both groups under study. Unequivocal results were found for waist circumference, in which case significant increase was observed only in patients without thromboembolic disease.

Table 2. Clinical characteristics of patients with thrombophilias – end of follow-up.

	History of VTE n = 51	**No history of VTE n = 45**	**p (χ^2 or t-test)**
Age, years (mean ± SD)	47.1 ±13.0	43.1 ± 12.9	0.14
Duration of follow-up, months (mean ± SD)	55.8 ± 8.0	56.5 ± 7.2	0.70
Male sex	18 (35.3)	14 (31.1)	0.83
History of cardiovascular disease	2 (3.9)	3 (7.3)	0.66
History of diabetes/impaired fasting glucose	2 (3.9)	2 (4.9)	1.00
Ever smoker	16 (35.2)	15 (33.3)	1.00
Treatment by statins	11 (21.6)	7 (15.9)	0.60
Antihypertensive treatment	15 (29.4)	8 (17.8)	0.22
Hormonal therapy	7 (13.7)	4 (18.9)	0.53
*Body mass index, kg*m^{-2}, (mean ± SD)*	27.2 ± 5.4	26.0 ± 4.7	0.19
Waist circumference, cm, (mean ± SD)	90.6 ± 16.5	88.6 ± 15.0	0.55
Systolic blood pressure, mm Hg, (mean ± SD)	125.7 ± 15.5	123.3 ± 18.6	0.49
Diastolic blood pressure, mm Hg, (mean ± SD)	78.8 ± 9.6	76.9 ± 10.6	0.36
Belcaro score above I (combined)	18 (53.0)	18 (46.2)	0.49
Belcaro score above I (carotid arteries)	19 (56.0)	17 (44.0)	0.30
Belcaro score above I (femoral arteries)	13 (38.2)	9 (23.1)	0.20

VTE = venous thromboembolism. Data are expressed as n (%) if not stated differently.

Table 3. Changes in risk factors in patients with thrombophilia with and without VTE.

	History of VTE n = 51	**No history of VTE n = 45**	**p*****
Change in mean Belcaro score combined above I (%)	-3%	0	0.403
Body mass index (kg/m^2)	1.03 ± 1.98 **	1.21 ± 1.67 **	0.663
Waist circumference (cm)	1.48 ± 7.67	4.11 ± 7.84 *	0.100
Systolic blood pressure (mm Hg)	3.10 ± 14.50	2.81 ± 12.01	0.916

Data are expressed as mean difference ± SD if not stated differently. * p<0.05, ** p<0.01; ***p = difference between two groups (χ^2 or t-test), VTE = venous thromboembolism.

Table 4. Changes in risk factors in patients with thrombophilia with and without VTE – gender oriented approach.

	Women		**Men**	
	History of VTE n=33	**No history of VTE n=31**	**History of VTE n=18**	**No history of VTE n=14**
Change in Belcaro score combined above I (%)	0	0	7.1	0
Body mass index (kg/m^2)	1.00 ± 2.14 *	1.16 ± 1.86 **	1.08 ± 1.7 *	1.33 ± 1.19 ***
Waist circumference (cm)	1.95 ± 8.88	3.24 ± 8.14 *	0.72 ± 5.17	5.92 ± 7.08 **
Systolic blood pressure (mm Hg)	1.40 ± 15.64	0.30 ± 12.20	6.11 ± 12.00*	8.61 ± 9.63 **

Data are expressed as mean difference ± SD if not stated differently. * p<0.05, ** p<0.01, *** p<0.001, VTE = venous thromboembolism.

The same results were obtained, when we excluded patients with the history of cardiovascular disease, diabetes mellitus and those treated by hypolipemic drugs, by antihypertensive drugs and by hormone therapy including steroids and estrogens. The statin treatment in group with a history of VTE increased from 5 to 11 patients. In contrast, it decreased from 9 to 7 patients in the group without a history of VTE. However, these differences were not statistically significant (data not shown).

In addition, we analyzed separately changes of preclinical atherosclerosis and risk factors in women and men to detect potential gender differences (Table 4). In this respect we observed non-significant increase in Belcaro score combined in men with a history of VTE. Regarding other parameters under study, similar results were obtained with the exception of systolic blood pressure, which significantly increased only in men. Nevertheless, differences in changes of studied factors between men and women were not statistically significant.

Discussion

In longitudinal prospective study, we did not observe difference in the progression of atherosclerosis between patients with thrombophilia depending on presence or absence of venous thromboembolic disease. We detected increase of all cardiovascular risk factors under study, which was similar in both groups. The increase of body mass index was significant in both groups, while waist circumference increased significantly only in patients without VTE. In addition, we have not found any gender differences in above studied parameters.

Venous thromboembolism is a serious complication of thrombophilias. Nevertheless, not all patients with established diagnosis of thrombophilia develop VTE (Crowther and Kelton 2003). Therefore, risk stratification for patients with thrombophilias could be of importance. In the past decade several studies indicated that preclinical atherosclerosis due its procoagulative action may be viewed as a potential risk factor for VTE (Prandoni *et al*. 2003, Hong *et al.* 2005). This may be of particular importance in patients with other significant hypercoagulable state such as thrombophilias, where non invasive detection of atherosclerosis could help in the stratification of the VTE risk.

In our previous cross-sectional study we found obesity and hypertension to be strongly associated with a history of venous thromboembolism in patients with thrombophilias. However, we did not find strong association between preclinical atherosclerosis and venous thromboembolism in this group of patients (Auzký *et al.* 2010) and these results did not significantly change during longitudinal follow-up. Although the prevalence of preclinical atherosclerosis was slightly higher in the VTE group – similarly as in previous study – there was no significant change of this parameter during the follow-up.

Several recently published studies also revealed negative results with respect to relation between VTE and atherosclerosis and its risk factors. In The Tromsø Study, carotid atherosclerosis did not prove a link between arterial and venous thrombosis (Hald *et al.* 2014) and traditional atherosclerotic risk factors, such as smoking, hypertension, dyslipidemia and diabetes mellitus were not risk factors for VTE (Brækkan *et al*. 2012). In the analysis from the Atherosclerosis Risk in Communities study elevated glucose was not related to VTE (Bell *et al.* 2013) and another study showed no association between variants of the lipoprotein(a) and VTE (Helgadottir *et al.* 2012). Thus preclinical atherosclerosis may not represent a strong risk factor of VTE and according to these results routine screening for atherosclerosis in the patients in the risk of VTE is not warranted. From our results these negative findings apply also for patients with thrombophilias and equally for men and women.

Major strength of our study includes the design of prospective longitudinal study with standardized protocol in patients with thrombophilias who are at particularly high risk of VTE and where development of better risk stratification systems could be more cost effective than in general population. In addition to traditional cardiovascular risk factors, we also focused on preclinical atherosclerosis as potential trigger for VTE.

Nevertheless, in addition to the possibility of absence of pathophysiological connection between VTE and atherosclerosis, these negative results could be caused by low number of patients and their relatively low risk status compared to the Czech population. More numerous population of patients with thrombophilias with more advanced atherosclerotic changes, which are at higher risk could reveal more robust association between VTE and atherosclerosis. Therefore, the limitations of our study include relatively small number of study subjects, relatively high rate lost for follow-up and inclusion of

carriers of MTHFR mutations who may not be at very high risk for VTE (Lijferin*get al.* 2007). In addition, in our study, no significant stenoses either in carotid or femoral arteries were detected and higher (combined) Belcaro score III was found only in 5 patients which could not represent the whole population of patients.

In conclusion, in longitudinal study of patients with thrombophilias we did not find strong association between preclinical atherosclerosis and its progression and venous thromboembolism even using gender oriented approach.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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