

Thrombophilia in venous leg ulcers: A comparative study in early and later onset

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ABSTRACT

Background: Several studies found that the patients with chronic venous ulcers (CVU) have an increased prevalence of thrombophilia (44-75%), similar to that observed in deep vein thrombosis (DVT). The patients who develop CVU before their 50th birthday appear to represent a distinct group in terms of etiology, natural history and prognosis. **Aim:** To analyze the nature and prevalence of thrombophilia in patients with early onset of CVU (before 50-years old) compared with a group of patients with later onset. **Methods:** Twenty-seven consecutive patients of each group were studied. They underwent clinical assessment and blood testing for factor V Leiden, prothrombin G20210A, methyltetrahydrofolate reductase C677T, plasminogen activator inhibitor type 1 (PAI-1) mutations, antithrombin, proteins C and S levels, and also antiphospholipid antibodies (anticardiolipin antibodies and lupus anticoagulant), cryoglobulins and cryoagglutinins. **Results:** All the patients had at least one thrombophilia. The prevalences of single, 2 and ≥ 3 thrombophilias were 29.6%, 40.7% and 29.6%, respectively, in the early onset group, compared with 33.3%, 59.2% and 7.4% in the later onset group. The PAI-1 4G/4G homozygous mutation was significantly more common in patients with early onset of ulcer. The prevalences of factor V Leiden, prothrombin G20210A, elevated titer of antiphospholipid antibodies and the presence of cryoglobulins were higher in the early onset group, although the differences were not statistically significant. **Conclusion:** Our study brings evidence of a higher thrombophilic risk among the patients with early onset of the CVU as they had significantly higher prevalence of multiple (≥ 3) thrombophilias ($P=0.03$), homozygous mutations ($P=0.03$) and family history of leg ulcer ($P=0.02$) when compared with patients with later onset. Thrombophilia screening is important in patients with CVU before the age of 50 in order to stratify the thrombotic risk and to allow an appropriate prophylactic and therapeutic management.

Keywords: Leg ulcer, thrombophilia, venous insufficiency

INTRODUCTION

Chronic venous ulcers (CVU) affect 1% of the adult population. They are associated with a marked reduction in the quality of life^[1] and consume 1-2% of the healthcare spending in most developed countries.^[2]

Thrombophilia consists of the predisposition to form clots inappropriately with consecutive venous thromboembolic events. It results from inherited or acquired factors or, more commonly, from their interaction.^[3] Inherited hypercoagulable factors include antithrombin, protein C, protein S deficiencies, factor V Leiden (resulting in activated protein C resistance - APCR), prothrombin G20210A^[3] and plasminogen activator inhibitor type 1 (PAI-1) mutations.^[4] Hyperhomocystinemia may be both an acquired and inherited abnormality, the most common form of genetic hyperhomocystinemia resulting from methyltetrahydrofolate reductase (MTHFR) C677T mutation.^[3] Acquired factors include antiphospholipid

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antibodies, immobility, trauma, postoperative state and malignancy.^[5] Cryoglobulins and cryoagglutinins are rare acquired abnormalities that may be associated with small vessel thrombosis.^[6]

Hypercoagulable disorders may cause ulceration as a consequence of postphlebotic syndrome following clinical and subclinical deep venous thrombosis (DVT), associated with microvascular thrombosis and the development of pericapillary fibrin cuff.^[7] Several studies found that the patients with CVU have an increased prevalence of thrombophilia (44-75%), similar to that observed in patients with DVT.^[8-10]

Most of CVU appear after the age of 65. MacKenzie *et al.*, observed that the patients who develop CVU before their 50th birthday appear to represent a distinct group in terms of etiology, natural history and prognosis.^[11] However, data comparing thrombophilia in early and late onset of CVU is lacking. The aim of this study is to analyze the nature and prevalence of thrombophilia in patients with early onset of CVU (before 50-years old) compared with a group of patients with later onset of CVU (after the age of 50).

METHODS

Consecutive patients with CVU observed at our Dermatology Department during a period of 13 months (June 2010-June 2011) were studied. Venous etiology of the ulcers was established on clinical grounds. All the patients had at least two of the following clinical features: Leg edema (51 patients), noticeable varicose veins (48 patients), hemosiderosis (25 patients), lipodermatosclerosis (11 patients), stasis eczema (10 patients) and *atrophiae blanche* (10 patients). The ulcers were located in the medial malleolar portion of the leg in 47 patients, with upward extension to the lower half of the leg in nine of them. Seven patients had their ulceration located in the external malleolar or anterior and lateral regions of the leg.

Patients who were undergoing anticoagulation therapy were excluded due to the known interference with thrombophilia testing. We also excluded patients with ulcers of other etiologies, such as diabetes (fasting plasma glucose ≥ 126 mg/dl) and arterial insufficiency, on base of positive history of intermittent claudication, ischemic cardiopathy or stroke, diminished or absent popliteal, posterior tibial and *dorsalis pedis* arteries pulses, unsatisfactory distal perfusion with capillary

nail refill time ≥ 3 seconds and positive Buerger test. Doppler study was not performed as clinical evaluation was considered sufficient to exclude significant peripheral arterial disease. Patients with leg ulcers caused by other disorders such as vasculitis, neoplasm, infection or pyoderma gangrenosum, diagnosed with biopsy, were excluded too. Twenty-seven patients with onset of the CVU before the age of 50 were included in the early onset group and 27 patients with onset of the CVU after the age of 50 formed the later onset group. History and clinical assessment was performed and blood was drawn for determination of inherited and acquired thrombophilia. Note was made of age, sex, history of venous thromboembolic event (VTE), history of precipitant factors for thrombosis (smoking, surgery, lower limb fracture, prolonged immobilization, malignancy) and history of a first-degree relative with leg ulcer. The patients underwent blood testing for APCR, factor V Leiden when APCR was less than 0.84 ($N > 0.84$), prothrombin G20210A, MTHFR and PAI-1 mutations, antithrombin, proteins C and S levels, and also antiphospholipid antibodies (anticardiolipin antibodies IgM and IgG, anti- β_2 -glycoprotein antibodies and lupus anticoagulant), cryoglobulins and cryoagglutinins. Homocysteine levels were tested only in the first 25 patients included (13 from the early and 12 from the later onset group), as the test was no longer available at our laboratory.

Antithrombin activity and protein C were measured using chromogenic assays (STA Antithrombin III and STA Protein C chromogen - DIAGNOSTICA STAGO, Roche Diagnostics®), with reference ranges of 80%-120% and 70-130%, respectively. A clotting assay was used to test protein S (STA Protein S Clotting - DIAGNOSTICA STAGO), with a normal range of 50-120% in female and 60-120% in male patients. Activated protein C resistance was determined with a clotting assay (APC Resistance - COATEST APC Resistance V - CHROMOGENIX®), with a normal range > 0.84 (normalized ratio). Prothrombin G20210A Kit and Factor V Leiden Kit, both from Roche Diagnostics®, and a LightCycler 2.0 instrument, were used to assess the two mutations. MTHFR C677T and PAI-1 mutations were determined using Lightcycler-DNA Master Hybridization probes from Roche Diagnostics® and primers and probes synthesized by TIB Molbiol®. The anticardiolipin and anti- β_2 -glycoprotein 1 antibodies IgM and IgG were detected using a MICRO-ELISA immunoenzymatic assay (QUANTA Lite™ ACA IgM and IgG and QUANTA

Lite™ β_2 GPI IgM and IgG, INOVA Diagnostics, Inc.). Pathological values are: IgG or IgM > 15 GPL-U/ml. Lupus anticoagulant was assessed using two clotting tests, both from American Diagnostica Inc.: DVVtest and DVV confirm. The lupus anticoagulant status was defined in relation to the normalized ratio (NR) between the two tests, as follows: Negative (NR < 1.2), weakly present (1.2-1.5) moderately present (1.5-2) and strongly present (>2). The cryoglobulins were detected by a serum precipitation reaction. It was considered positive when precipitation occurred in a sample stored at 4°C, while the control sample stored at room temperature showed no reaction. The detection and titration of cryoagglutinins was performed according to the methods described in the Technical Manual of American Association of Blood Banks.^[12] A titer of greater than 1:64 was considered abnormal. Homocysteine was measured using an immunoassay from Abbot Laboratories, with a normal range of 5.0–15.0 μ mol/L.

Information about the nature of the tests was given, using the local language. Consent was obtained from all of the patients, being witnessed by the first two authors. They were told about the possible usefulness of the tests in explaining the development of the leg ulcers, but that results might not affect their subsequent treatment. The tests were performed and supported by the hospital, as being part of the procedure for our patients, when needed.

Statistical analysis of the data was performed using the SPSS19 software. The χ^2 -test was used to determine the two-tailed statistical significance of differences between proportions in 2×2 tables (categorical variables). A *P* value of less than 0.05 was considered significant.

RESULTS

There were 27 patients in the early onset group (12 men and 15 women), with a mean actual age of 57.6 years (range 23-81) and 27 patients in the later onset group (7 men and 20 women) with a mean actual age of 70 years (range 55-84). The average age of ulcer onset was of 36 years (range 18-49) in the early and of 62 years (average 50-77) in the later onset group.

All the patients had at least one thrombophilia. The prevalence of single, 2 and ≥ 3 thrombophilias were 29.6%, 40.7% and 29.6%, respectively in the early

onset group, compared with 33.3%, 59.2% and 7.4% in the later onset group [Figure 1]. The early onset group had significantly more patients with ≥ 3 thrombophilias ($n=8$) than the control group ($n=2$) ($P=0.03$). The total number of homozygous mutations (including PAI-1 4G/4G genotype, MTHFR and prothrombin G20210A mutations) was significantly higher in the early onset group ($n=19$) compared with the other group ($n=11$) ($P=0.03$).

The individual thrombophilia data are summarized in the Table 1 below. The prevalences of factor V Leiden, prothrombin G20210A, elevated titer of

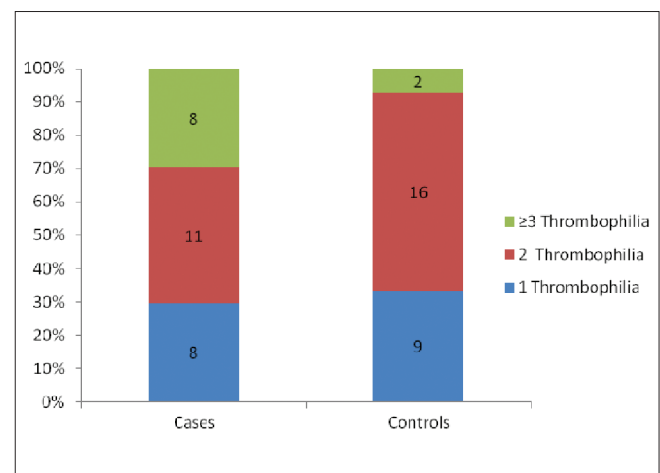


Figure 1: Prevalence of thrombophilia in patients with onset of chronic leg ulcer before 50 years old (cases) compared patients with onset of CLU after the age of 50 (controls)

Table 1: Individual thrombophilia data

Variable	Cases N (%)	Controls N (%)
Factor V Leiden		
heterozygote	1 (3.7)	0
Prothrombin G20210A mutation	3 (11.1)	2 (7.4)
homozygote	1 (3.7)	0
heterozygote	2 (7.4)	2 (7.4)
PAI-1 polymorphism	23 (85.1)	26 (96.2)
homozygote	16 (59.2)	8 (29.6)
heterozygote	7 (25.9)	18 (66.6)
MTHFR mutation	16 (59.2)	17 (62.9)
homozygote	5 (18.5)	3 (11.1)
heterozygote	11 (40.7)	14 (51.8)
Protein C $\leq 70\%$ activity	0	0
Protein S $\leq 60\%$ activity	0	0
Antithrombin $\leq 70\%$ activity	0	0
Anticardiolipin antibodies	3 (11.1)	2 (7.4)
Lupus anticoagulant	4 (14.8)	1 (3.7)
Cryoglobulins	2 (7.4)	0
Cryoagglutinins titer ≥ 64	2 (7.4)	2 (7.4)

PAI-1: Plasminogen activator inhibitor type 1, MTHFR: Methyltetrahydrofolate reductase

antiphospholipid antibodies and the presence of cryoglobulins were higher in the early onset group, while the overall prevalences of MTHFR mutation and PAI-1 4G allele (including heterozygotes and homozygotes) were higher in the later onset group, although the differences were not statistically significant. Specifically, MTHFR mutation was found in 16 (59.2%) patients from the early onset group and in 17 (62.9%) in the later onset group; PAI-1 4G allele was found in 23 (85.1%) patients from the early onset group and in 26 (96.2%) in the later onset group. The PAI-1 4G/4G genotype was significantly more common in patients with onset of ulcer before the age of 50 ($n=16$) compared with later onset ($n=8$) ($P=0.03$).

No cases of antithrombin, protein C and protein S deficiency were found in our series.

High levels of homocysteine ($\geq 15 \mu\text{mol/L}$) were found in 9 (36%) of the 25 patients tested (2 from the early and 7 from the later onset group) and eight of them had the MTHFR mutation (3 homozygotes and 5 heterozygotes).

History of CVU in a first-degree relative was found in 11 patients in the early onset group compared with only four patients from the other group and this difference was significant ($P=0.02$). History of DVT was present in eight patients from the early onset group (two of them with recurrent episodes) and six patients from the later onset group (one of them with recurrent episodes). Precipitant factors for thrombosis before the ulcer onset were identified in eight patients from the early onset group (three patients with lower limb fracture, three heavy smokers and two patients with prolonged immobilization) and three patients from the later onset group (lower limb fracture).

DISCUSSION

Our study found that multiple (≥ 3) thrombophilias and homozygous mutations were significantly more common in patients with onset of CVU before the age of 50 comparing with later onset. When ≥ 2 factors were taken into consideration, no difference was found between early (19/27) and later onset (18/27) groups. Only the coexistence of ≥ 3 factors was significant.

Individuals with more than one thrombophilia (homozygotes or compound heterozygotes) have an increased risk of venous thromboembolic events

(VTE).^[13] Patients who develop venous leg ulceration before their 50th birthday are more likely to have a history of DVT,^[11] which, in turn, is recognized as a major risk factor for intractable chronic venous ulcers.^[7] The higher tendency for a hypercoagulable state in the case group may explain the onset of ulceration earlier in life.

All our patients had at least one thrombophilia. The reported prevalence of at least one thrombophilia in CVU was of 41%^[6] and 75%^[7] in two studies not including the MTHFR and PAI-1 mutations and of 70% in another study which included MTHFR mutation but not the PAI-1 polymorphism.^[10] The highest prevalence (100%) of at least one thrombophilia found in our study may be explained by the inclusion of the PAI-1 polymorphism and MTHFR mutation in the hypercoagulability testing.

PAI-1 is the central component of the fibrinolytic system. The 4G allele is associated with elevated levels of PAI-1,^[14] which are higher in homozygotes than heterozygotes,^[15] and appears to increase the risk of venous thrombosis, particularly in subjects with other genetic thrombophilic defects.^[4] The prevalence in the general population was reported to be of 18.4% for the 4G/4G genotype and of 44.2% for the 4G/5G genotype.^[15] In a population living in the same geographical region as ours (North of Portugal) the frequency of PAI-1 4G allele was of 76% in patients with a history of DVT before the age of 40 years and of 68% in healthy controls, this value being superior to other reports.^[16] Our study has shown a very high prevalence of 4G allele in CVU patients (49 of 54 patients; 90.7%), and a significantly higher prevalence of homozygotes in patients with onset of CVU before the age of 50 years compared with later onset.

The C677T polymorphism of the MTHFR gene is relatively common in general population. About 34-37% of the US Caucasians are heterozygous for this variant and 12% are homozygous.^[13] It has been associated with slight elevations of homocysteine levels, especially when folate deficiency is present; hyperhomocysteinemia in turn increases the risk of VTE.^[13] Our study revealed a prevalence of the C677T polymorphism of 61% in patients with CVU, higher than reported in general population, but no significant difference was found between the early and later onset groups.

Factor V Leiden is present in 5% of the white population and in 7.7-36% of the CVU patients;^[9] in this study it was found in one patient with early onset of the CVU.

Prothrombin G20210A mutation is present in 2% of the white population and has been reported in 0-4% of the CVU patients.^[9] One study performed in a population from the same geographical area of Portugal found higher prevalence of heterozygosity for the G20210A allele, namely of 5% in healthy population and of 12.5% in patients with a history of DVT before 40 years old.^[17] In our study this mutation was present in five out of 54 patients with CVU (9.2%); 3 of them, including the only homozygote patient, had the onset of the ulcer before the age of 50.

Antithrombin, protein C and protein S deficiencies, are established genetic factors for thrombophilia^[3] and were found in percentages of 11%, 8% and 13%, respectively in a large cohort of CVU patients.^[18] Our study did not reveal deficiencies of antithrombin, protein C and protein S, probably due to the smaller number of patients and regional particularities of the population. Another possible explanation is that patients carrying these defects may be under anticoagulation treatment and so excluded from the study.

The antiphospholipid antibodies are present in 10-20% of the population and have been reported in 5.6-59% of the CVU patients.^[9] A large study of 310 CVU patients did not find significant difference concerning the antiphospholipid antibodies between the groups with or without post-thrombotic syndrome.^[18] Ten out of 54 patients (18.5%) in the present series had elevated anticardiolipin antibodies or positive lupus anticoagulant, most of them (7) having the onset of the ulcer before 50 years old.

Cryoglobulins and cryoagglutinins are immunoglobulins that precipitate at cold temperatures and may cause vaso-occlusive phenomena. They constitute rare causes of leg ulceration.^[19,20] Two patients in the early onset group had positive cryoglobulins, but none in the later onset group, while cryoagglutinins were found in two patients of each group.

The presence of a single heterozygote thrombophilia does not confer a significant increase in the risk of thrombosis, but when more such abnormalities coexist and are accompanied by acquired thrombophilia and

precipitant factors, then the risk of DVT increases dramatically.^[7] These patients should avoid smoking or oral contraceptive, may receive prophylactic hypocoagulation when precipitant factors are added (surgery, immobilization), or may be candidates for prolonged hypocoagulation after a VTE.^[13]

Regarding the management of the CVU in patients with thrombophilia, several reports have found beneficial effects of warfarin, dalteparin, clopidogrel, aspirin, or combination aspirin-pentoxifylline.^[9,21,22]

CONCLUSIONS

Patients with onset of the CVU before the age of 50 have significantly higher prevalence of multiple (≥ 3) thrombophilias, homozygous mutations and family history of leg ulcer when compared with patients with later onset. The PAI-1 mutation, which was not tested before in CVU patients, had a significantly higher prevalence of homozygotes in patients with early onset. Our data bring new evidence of a higher thrombophilic risk in the patients with early onset of CVU, supporting the hypothesis that these patients represent a distinct group. Thrombophilia screening is important in patients with CVU before the age of 50 in order to stratify the thrombotic risk and to allow an appropriate prophylactic and therapeutic management. Future studies will establish the paper of antiaggregant or anticoagulant therapy in CVU associated with thrombophilia.

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