# Risk factors and recurrent thrombotic episodes in patients with cerebral venous thrombosis

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**Background.** The prevalence of thrombophilic abnormalities in patients with cerebral vein thrombosis has been reported to be similar to that in patients with deep vein thrombosis of the lower limb. The role of gender-specific risk factors (pregnancy, oral contraceptives) is well established, whereas that of other acquired risk conditions is debated.

**Materials and methods.** We screened 56 patients with cerebral vein thrombosis and 184 age- and sex-matched apparently healthy controls for prothrombin (factor II, FII) G20210A and factor V Leiden polymorphisms; protein S, protein C, and antithrombin deficiency; anticardiolipin antibodies; hyperhomocysteinaemia and other putative risk factors.

**Results.** The G20210A polymorphism was found in 29.1% of patients and in 5.7% of controls (odds ratio [OR] 7.1; P <0.0001; adjusted OR 12.67, P <0.0001). Frequencies of factor V Leiden and hyperhomocysteinaemia were not significantly different in patients and controls, nor were the other thrombophilic tests and some established cardiovascular risk factors, such as smoking, obesity or overweight and arterial hypertension. Conversely, 53.7% of the women who developed cerebral vein thrombosis did so while assuming oral contraceptives (OR 6.12; P <0.0001), with a further increase of risk in FII G20210A carriers (OR 48.533). Some associated diseases (onco-haematological disorders and infections) also had a significant role. Over a median 7-year follow-up, irrespective of the duration of antithrombotic treatment, 9/56 (16%) patients had further episodes of venous/arterial thrombosis. No significant risk factor for recurrent thrombosis was identified.

**Discussion.** In spite of the limitations of the sample size, our data confirm the role of FII G20210A mutation in this setting and its interactions with acquired risk factors such as oral contraceptives, also highlighting the risk of recurrent thrombosis in cerebral vein thrombosis patients.

**Keywords:** risk factors, cerebral venous thrombosis, recurrences.



## Introduction

Cerebral vein thrombosis (CVT) is a rare but potentially fatal cause of acute stroke, historically associated with infections (otomastoid, orbital, cutaneous). After the advent of antibiotics, cancer, pregnancy/puerperium, systemic diseases, dehydration, intracranial tumours, oral contraceptives (OC) and thrombophilia became the most commonly identified predisposing conditions. CVT has also been reported in association with fibrous thyroiditis, jugular thrombosis after catheterisation, and idiopathic jugular vein stenosis. Other risk factors include surgery, head trauma, arterio-venous malformations, and autoimmune disorders[1]. However, in up to 30% of cases, no underlying aetiology or associated condition can be found[1].

The annual incidence of CVT is estimated to be 2-4 cases per million adults and 7 cases per million neonates and children[2-4]. The rarity of such venous thromboses may be explained by the lesser pathophysiological impact of venous stasis, because of the absence of valves in the cerebral veins[1-6]. Based on epidemiological data, compared with arterial ischaemic stroke, CVT more frequently affects young adults and is more common in women than in men (3:1 ratio)[2], OC and pregnancy/puerperium being well established gender-specific transient risk factors[2]. In a recent international, retrospective cohort study of 706 patients with a first CVT conducted by Dentali *et al.*, 73.7% of patients were females[7]. Similar figures were reported in the multicentre, prospective International Study on Cerebral Vein and dural sinus Thrombosis (ISCVT), in which clinical presentation, risk factors and outcome of 624 adult patients with symptomatic CVT were analysed[5]. In that study, 465 (74.5%) patients were females, and male sex was a predictor of death or dependence (defined by a score of 3-5 on the modified Rankin scale) in the multivariate analysis[5]. A more recent sub-analysis of the data from the ISCVT study

showed that women were significantly younger and more frequently had an acute onset of symptoms, with headache at presentation. A gender-specific risk factor (i.e. OC use, pregnancy, puerperium, or hormonal replacement therapy) was found in 65% of women. However, female patients had a better prognosis than males (complete recovery in 81% *vs* 71%; P =0.01), which was entirely due to patients with gender-specific risk factors. The clinical presentation, risk factor profile, and outcome of women without gender-specific risk factors were similar to those of men. Logistic regression analysis confirmed that the absence of gender-specific risk factors was a strong and independent predictor of poor outcome in women with CVT (OR, 3.7; 95% confidence interval [CI], 1.9-7.4)[2].

A high prevalence of thrombophilic abnormalities, similar to that found in patients with deep vein thrombosis of the lower limb, has been reported in patients with CVT[1,2,4,8-10]. The risk of CVT has been calculated to be 10-fold and 22-fold increased in patients carrying the factor II (FII, prothrombin) G20210A gene mutation or using OC, respectively, with an exponentiation of risk (150-fold) when both conditions coexisted[8]. Hyperhomocysteinaemia has also been shown to be associated with a 4-fold increased risk of CVT[11]. There are some data suggesting that established cardiovascular risk factors (cigarette smoking, obesity, arterial hypertension, diabetes, hypercholesterolaemia) are also risk factors for venous thrombosis, but the associations are still controversial[12].

On this background, our study was aimed to evaluate the role of inherited and acquired thrombophilic abnormalities and of other putative risk factors (smoking habit, obesity or overweight, arterial hypertension, hypercholesterolaemia, infections and onco-haematological or autoimmune diseases) in a cohort of consecutive patients with CVT. Recurrent thrombotic events were also analysed over a median 7-year follow-up.

## Materials and methods

### Study population

In a retrospective-prospective open study, we enrolled 56 consecutive inpatients (15 men and 41 women; mean age 34.98±11.02 years), referred to our tertiary care Centre from 1997 to 2012, because of an episode of CVT. One hundred and eighty-four age-and sex-matched apparently healthy subjects (mean age 35.05±11.33 years), from the same ethnic background (friends and partners of patients) served as controls (Table I). The study was approved by the Ethical Committee for Human Studies of our Institution and written informed consent was obtained from all participants (controls and patients).

### Laboratory testing and collection of clinical data

All participants were screened for inherited and acquired thrombophilic abnormalities, including FII G20210A and factor V G1691A (FV Leiden) polymorphisms, protein S, protein C and antithrombin deficiency, anticardiolipin antibodies, lupus anticoagulant (LAC) and hyperhomocysteinaemia. Moreover, clinical records were carefully examined by trained staff in order to identify cardiovascular risk factors and other possible associated conditions.

At enrolment, for each subject, blood was collected from an antecubital vein with minimal stasis into dry (5 mL) and citrate-containing (15 mL) tubes (Vacutainer

**Table I -** Clinical characteristics and prevalence of risk factors in the study population.

| Variable | CVT patients (n, %) | Controls (n, %) | OR (95% CI) | P^ |
|---|---|---|---|---|
| Gender (F/M) | 41/15 | 134/50 | | NS |
| Age (mean±SD) | 34.98±11.02 | 35.05±11.33 | | NS |
| FII G201210A | 16/55 (29.1%) | 10/183 (5.5%) | 7.09 (2.99-16.82) | <0.0001 |
| FV Leiden | 3/56 (5.4) | 15/183 (8.2%) | 0.6 (0.17-2.274) | NS |
| Antiphospholipid antibodies* | 2/47 (4.3%) | 5/113 (4.4%) | | NS |
| Infectious diseases | 7/55 (12.7%) | 2/183 (1.1%) | 13.19 (2.65-65.59) | <0.001 |
| Cancer and haematological disorders | 7/54 (13%) | 4/183 (2.2%) | 6.66 (1.87-23.72) | 0.003 |
| Oral contraceptive users | 22/41 (53.7%) | 21/132 (15.9%) | 6.12 (2.83-13.23) | <0.0001 |
| Autoimmune diseases | 5/55 (9.1%) | 7/182 (3.8%) | 2.5 (0.76-8.21) | NS |
| Arterial hypertension | 9/56 (16.1%) | 27/182 (14.8%) | 1.09 (0.40-2.50) | NS |
| Hypercholesterolaemia | 23/49 (46.9%) | 46/159 (28.9%) | 2.17 (1.12-4.19) | 0.024 |
| Hyperhomocysteinaemia | 15/44 (34.1%) | 44/148 (29.7%) | 1.22 (0.59-2.50) | NS |
| Obesity (BMI≥30) | 19/40 (47.5%) | 56/144 (38.9%) | 1.42 (0.70-2.87) | NS |
| Cigarette smoking | 26/55 (47.3%) | 67/183 (36.6%) | 1.52 (0.80-2.80) | NS |

**Legend**
BMI: body mass index; CI: confidence interval; CVT: cerebral vein thrombosis; FII: prothrombin; FV: factor V; NS: not significant; OR: odds ratio.
^: univariate analysis; at multivariate logistic regression all significant variables were confirmed, with the exception of hypercholesterolaemia.
*: including lupus anticoagulant and/or anticardiolipin antibodies.

BD, Franklin Lakes, New Jersey, USA) and processed immediately. Gene polymorphisms were detected through a polymerase chain reaction technique on DNA extracted from leucocytes[13], using previously described primers and experimental conditions[14,15]. Plasma antithrombin levels were determined chromogenically in a Sysmex CA-6000 coagulometer (DASIT, Milan, Italy), employing a commercially available reagent (Antitrombina III DASIT, Milan, Italy). Serum homocysteine (Axis Homocysteine EIA, Bio-Rad Diagnostics, Segrate, Italy) and plasma protein S (Asserachrom Protein S, Boehringer Mannheim, Germany) were measured with an immunoenzymatic assay, whereas plasma protein C was assayed with a chromogenic method (Behrichrom Protein C, Behring Diagnostics Gmbh, Marburg, Germany). LAC was detected by specific activated partial thromboplastin time-derived assays (Hemosil LAC Confirm, Instrumentation Laboratory, Milan, Italy) and anticardiolipin antibodies (ACA) were measured by an immunoenzymatic assay (Coaliza Anti-Cardiolipin, Instrumentation Laboratory). The presence of LAC and/or ACA was confirmed in at least one other assessment 8-12 weeks later, in the absence of clear-cut infection or inflammatory disease[16].

Hyperhomocysteinaemia was defined by fasting total homocysteine levels exceeding the 95$^{th}$ percentile of the sex-specific distribution (15.0 μmol/L for men and 12 μmol/L for women) in a control population of healthy subjects from the same geographic area[17]. Participants were classified as hypertensive if they had a resting blood pressure >140 mmHg (systolic) and/or >90 mmHg (diastolic) on repeated measurements over 3-6 months or if they were on antihypertensive medications. Individuals were considered diabetic if they had a fasting plasma glucose level ≥1.4 mM, or if they were on hypoglycaemic medications; hypercholesterolaemia and hypertriglyceridaemia were defined as a fasting plasma cholesterol ≥5.2 mM or triglyceride level ≥1.7 mM, respectively, confirmed by at least another previous evaluation over a 2-year period, or treatment with lipid-lowering medications. Smoking habit was recorded for current smokers and smokers who had ceased smoking less than 1 year previously. Body mass index was estimated according to the formula:

$$\frac{\text{body weight [kg]}}{\text{body height [m]}^2};$$

individuals were considered overweight or obese if their body mass index was ≥25 or 30, respectively. The use of OC in women was carefully investigated. OC users were considered those on current treatment or with previous use within 1 month before entering the study.

**Statistical analysis**

The Statistical Package for Social Science (SPSS 10.0 for Windows) was employed for the statistical analysis. Patients' clinical characteristics were compared with the independent sample t-test for continuous variables and the chi-squared test for categorical dichotomous variables. The odds ratio (OR) and 95% confidential intervals (CI) were calculated in each case. In a linear regression (stepwise) model with CVT (presence/absence) as the dependent variable, the role of age, gender, smoking habit, obesity, being overweight, arterial hypertension, hypercholesterolaemia, thrombophilic abnormalities, infections and onco-haematological or autoimmune diseases was evaluated as independent variables. Gender-specific risk factors (pregnancy/puerperium and OC use) were also considered in the female study population. In each analysis, two-tailed probability (P) values <0.05 were considered statistically significant.

## Results

In our study population, 16/55 (29.1%) CVT patients had the FII G20210A polymorphism (including a single case of homozygous AA genotype) compared with 5.7% of controls (OR 7.1, 95% CI 2.99-16.82; P <0.0001; adjusted OR 12.67, P <0.0001). The frequency of FV Leiden was 5.4% in patients and 8.2% in controls (P >0.05) (Table I). One patient and one healthy subject carried both the FII and the FV variants. No natural anticoagulant protein (antithrombin, protein C, protein S) deficiency was found. Hyperhomocysteinaemia was diagnosed in comparable numbers of patients and controls (34.1% and 29.7%, respectively; P >0.05); likewise, there were no significant differences in the presence of LAC or ACA between the two groups (Table I).

Among the common cardiovascular risk factors, smoking habit, obesity/being overweight and arterial hypertension showed similar prevalences in CVT patients and controls, whereas hypercholesterolaemia was significantly more frequent among the patients (46.9% of patients *vs* 28.9% of controls; OR 2.17, 95% CI 1.13-4.19; P <0.02) (Table I). However, the statistical significance was no longer found in the logistic regression model.

As far as regards gender-specific risk factors, CVT occurred in 12.5% of women during pregnancy/puerperium and, interestingly, in 22/41 (53.7%) while assuming OC. This prevalence was significantly higher than that of contraceptive users among control women (21/132, 15.9%, P <0.0001, OR 6.12; 95% CI 2.83-13.23). OC assumption in carriers of the FII G20210A polymorphism resulted in a further increase of risk of CVT (OR 48.53; 95% CI 5.57-422.563; P <0.001), whereas no influence was detectable in patients carrying FV Leiden.

Among associated conditions, the prevalence of oncological and haematological disorders (a case of osteosarcoma, five cases of chronic myeloproliferative disease, and a patient with paroxysmal nocturnal haemoglobinuria, previously reported[18]) was significantly higher in patients than in controls and associated with an increased risk of CVT (7/54, 13.0% *vs* 4/183, 2.2%; OR 6.66; 95% CI 1.87-23.72; P=0.003). The findings regarding the prevalence of infectious diseases were similar (active hepatitis, pneumonia or encephalitis; 7/55, 12.7% *vs* 2/183, 1.1%; OR 13.19; 95% CI 2.65-65.59; P <0.001). Autoimmune disorders (thyroiditis and two cases of inflammatory bowel disease) were also detected more frequently among patients than among controls (5/55, 9.1% *vs* 7/182, 3.8%), but this difference did not reach statistical significance (Table I).

No inherited thrombophilic abnormality or acquired risk factors were recognized in three CVT patients (5.4%).

According to the logistic regression (stepwise) model, the adjusted OR for FII G20120A was 12.67 (P <0.0001). The presence of infections was also significantly correlated with the risk of CVT in the multivariate analysis (adjusted OR 20.86, P <0.009). Onco-haematological disorders were associated with an approximately 6-fold increased risk of CVT (adjusted OR 5.77; P <0.017), whereas the other variables considered were not statistically significant in the multivariate analysis.

### Recurrence of thrombosis

Patients were followed up for a median of 7 years (range, 1 to 15 years). Irrespective of the duration of antithrombotic treatment, no CVT recurrence occurred. However, in this cohort of CVT patients, 9/56 (16%) developed an objectively diagnosed further episode of venous or arterial thrombosis, i.e. three cases of lower limb deep vein thrombosis, two cases of upper limb deep vein thrombosis, one case of superficial venous thrombosis of the lower limb, one case of portal vein and mesenteric thrombosis, one case of Budd-Chiari syndrome[18], and a case of transient ischemic attack. Of this nine patients, three experienced other thrombotic episodes also prior to the occurrence of CVT. Moreover, nine patients had a history of venous or arterial thrombotic episodes in other sites prior to the occurrence of CVT. Previous thrombotic events included five lower limb deep vein thromboses, a recurrent left eye central vein retinal occlusion, a myocardial infarct, and an ischaemic stroke.

Overall, 14/56 (25%) patients (64% females) had multiple thrombotic episodes (Table II). Four out of these patients (28.6%) had thrombophilia (severe in one case, mild in three), three suffered from haematological diseases, and one patient had chronic inflammatory bowel disease. However, no significant risk factor for recurrent thrombosis was identified when clinical characteristics (pregnancy/puerperium, arterial hypertension, hypercholesterolaemia, hyperhomocysteinaemia, obesity/being overweight, cancer, autoimmune disorders infections, surgery) and the presence of thrombophilia were compared between patients with and without multiple thrombotic events.

## Discussion

In this cohort of 56 consecutive patients with CVT, the analysis of risk factors confirms the role of the FII G20210A polymorphism and the importance of acquired risk factors such as OC, also highlighting the enhanced risk conferred by the interaction of these factors, i.e. in women carriers of FII G20210A polymorphism who assume OC[8]. At variance with the findings of other studies[11,19,20], in our cohort patients

**Table II -** Clinical features of CVT patients with multiple thrombotic events°.

| Case | Sex | FII G20210A | FV Leiden | Onco-haematological disorders | Events prior to CVT | Events after CVT |
|---|---|---|---|---|---|---|
| 1 | F | Heterozygous | Negative | Yes | LL DVT | UL DVT |
| 2 | F | Heterozygous | Negative | No | LL DVT + PE | - |
| 3 | M | Heterozygous | Heterozygous | Yes | LL DVT | Portal + mesenteric vein |
| 4 | M | Heterozygous | Negative | No | MI | - |
| 5 | M | Negative | Negative | No | - | LL DVT |
| 6 | M | Negative | Negative | No | - | UL DVT |
| 7 | M | Negative | Negative | No | - | LL DVT |
| 8 | F | Negative | Negative | No | LL DVT + PE | LL DVT; TIA |
| 9 | F | NA | Negative | No | - | LL SVT |
| 10 | F | Negative | Negative | No | Stroke | - |
| 11 | F | Negative | Negative | No | LL DVT | - |
| 12 | F | Negative | Negative | No | - | LL DVT |
| 13 | F | Negative | Negative | No | RVO | - |
| 14 | F | Negative | Negative | Yes | - | Budd-Chiari |

**Legend**
CVT: cerebral vein thrombosis; DVT: deep vein thrombosis; LL: lower limb; MI: myocardial infarction; NA: not available; PE: pulmonary embolism; RVO: retinal vein occlusion; TIA: transient ischaemic attack; UL: upper limb.
°No patient had history of infectious or autoimmune disease.

carrying the FV Leiden polymorphism did not show an increased risk of thrombosis, even in the case of women on OC, nor was a role for other thrombophilic abnormalities found. In spite of the limitations of a relatively small, single-centre, study population, a significant contribution of thrombophilia in the pathogenesis of CVT was confirmed, but according to our data it is likely to be limited to the FII polymorphism. On the whole, our data are consistent with those from the large cohorts of CVT patients recently reported in multicentre studies (Table III), which contributed significantly to the knowledge about these rare venous thrombotic events.

A role for other associated conditions hardly emerges in this analysis, particularly for infectious diseases and onco-haematological disorders. The possible occurrence of CVT as a complication of otomastoiditis, otitis, encephalitis and/or meningitis has been widely established[1]. However, despite the statistical correlation, the small sample size and the heterogeneity of clinical presentations in our study do not enable definite conclusions to be drawn concerning the role of specific sites of infectious diseases. None of our patients experienced otomastoidal infections and only one had encephalitis. However, our data support the role of an inflammatory-related hypercoagulable state due to underlying infectious diseases. Onco-haematological disorders (mainly chronic myeloproliferative diseases) were associated with an approximately 6-fold increase of risk of CVT in our analysis. The relationship between haematological diseases and thrombosis has been extensively reported, even in association with the Janus kinase 2 (JAK2) V617F mutation. De Stefano *et al.*[21] reported an increased risk of atypical site venous thrombosis in patients with myeloproliferative neoplasms younger than 60 years carrying the JAK2 V167F mutation. The association between the JAK2 V617F variant and thrombosis in atypical sites was maintained even in patients without overt haematological disorders at diagnosis of the thrombotic event, which was mostly splanchnic vein thrombosis. We were not able to evaluate this relationship because JAK2 polymorphism was not routinely searched for in our patients.

Although no specific data concerning the prevention of CVT are available, the possible contribution of acquired conditions in the pathogenesis of CVT further highlights the need for adequate thromboprophylaxis[22] in patients with infectious/inflammatory diseases and haematological disorders, which is often overlooked. Beyond primary prophylaxis, strategies for preventing recurrence of thrombosis should be carefully taken into account in patients with a previous history of thrombotic episodes, including CVT, as shown by our data and other recent studies in the literature[5,7,23,24]. Over a median follow-up of 7 years, 9/56 (16%) patients developed thrombotic recurrences in our cohort, in most cases DVT, but venous thromboses in atypical sites were also reported. This rate of recurrence, irrespective of the duration of antithrombotic treatment, was higher than that reported by Martinelli *et al.* (10%), and by other authors (Table III)[5,7,25], over a long-lasting follow-up. However, we were not able to identify risk factors for recurrence in our population. In this respect, conflicting data are available in the literature. In a retrospective study, the duration of oral anticoagulation and classical thrombophilic abnormalities did not predict recurrences, whereas male sex, discontinuation of anticoagulation (mostly in the first year) and thrombophilia (particularly severe thrombophilic defects, such as homozygous or compound heterozygous polymorphisms, and natural anticoagulant deficiencies), were related to a higher rate of recurrence[25].

In conclusion, although the occurrence of these severe, often life-threatening thrombotic events remain poorly predictable, our study contributes to identify clinical conditions associated with the risk of CVT, and confirms the role of thrombophilia, particularly of the FII G20210A polymorphism, in the setting of venous thrombosis in unusual sites. The clinical implications of thrombophilia are often disputed[26,27]; in this respect, the possible influence on some relevant issues in CVT patients (duration of anticoagulation and, particularly, a not negligible risk of recurrent thrombosis) should be further investigated.

**Table III -** Comparison of data from this study and previous multicentre CVT cohorts.

| Study | Design | CVT patients N | FII G20210A frequency (%) | FV Leiden frequency (%) | Follow-up (median) Months | CVT recurrences N (%) | All thrombotic recurrences N (%) |
|---|---|---|---|---|---|---|---|
| This study | Retrospective-prospective, open, case-control | 56 | 29.1% | 5.4% | 84 | 0% | 9/56 (16%) |
| Ferro JM, *et al.* Stroke 2004[5] | Prospective, observational, multinational | 624 | NA* | NA* | 16 | 14/624 (2.2%) | 27/624 (4.3%) |
| Dentali F, *et al.* J Thromb Haemost 2012[7] | Retrospective, international | 706 | 19.1% | 9.1% | 40 | 31/706 (4.4%) | 46/706 (6.5%) |

**Legend**
CVT: cerebral vein thrombosis; NA: not available.
*Total genetic thrombophilias: 22.4%

*The Authors declare no conflicts of interest.*

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