The role of coagulation disorders in patients with retinal vein occlusion

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Summary

Background: The role of a hypercoagulable state in the pathogenesis of retinal vein occlusion (RVO) has not been conclusively established.
Aim: To analyse the prevalence of thrombophilia in RVO.
Design: Prospective case–control study.
Methods: All the patients diagnosed with RVO were referred to an Internal Medicine clinic and compared with sex- and age-matched individuals from a population-based cohort. Demographic, clinical and laboratory variables (including a thrombophilia panel) were analysed.
Results: One hundred and seventy patients (93 men and 77 women; 68 ± 11 years) and 170 controls (80 men and 90 women; 67 ± 10 years) were included. RVO was peripheral in 113 cases. Genetic thrombophilia was detected in 13% of patients. Acquired thrombophilia was observed in 10% of cases and 4.7% of controls (P < 0.01). Sixty-three percent of cases and 24.6% of controls had serum hyperhomocysteinemia (odds ratio [OR] 5.2, IC 95% 2.7–10.1; P < 0.0001). In RVO patients aged <50 years (n = 11), 36.4% had genetic thrombophilia (P = 0.04), as well as 50% of those without vascular risk factors (n = 18; P = 0.01). Forty-one (24%) patients with RVO received antiplatelet agents and 13 (7.6%) were on anticoagulants due to preexistent atrial fibrillation.
Conclusions: We suggest that, in patients with RVO, hyperhomocysteinemia and antiphospholipid syndrome should be ruled out. Moreover, a study of genetic thrombophilia should only be considered in those aged <50 years or without cardiovascular risk factors. Antiplatelet therapy with aspirin is probably the treatment of choice of RVO, to reduce the overall vascular risk. Anticoagulation should only be considered in patients with high-risk thrombophilia.

Introduction

Retinal vein occlusion (RVO) is the second leading cause of retinal vascular impairment, after diabetic retinopathy, and it entails an increase in cardiovascular mortality.¹⁻³ The mean age at onset is around 65 years, and this disorder affects 2.1/1000 patients aged 40 years or older and 5.4/1000 patients more than 64 years.⁴ Depending on the location of venous blockage, RVO is classified as central RVO or peripheral/branch RVO, which is four times more frequent than the central type.⁵⁻⁶ Classical vascular risk factors (VRFs), such as high blood pressure, dyslipidemia, diabetes mellitus and smoking, represent the main etiopathogenic factors for RVO. Therefore, in this context, RVO may be considered as a manifestation of systemic atherosclerosis.⁷⁻⁹
On the other hand, the role of a hypercoagulable state in patients with RVO has not been conclusively established. In this sense, the largest meta-analysis, published to date, found a clear association between RVO and hyperhomocysteinemia or antiphospholipid syndrome (APS) and, to a lesser extent, with the presence of FV:Q506 and FII:G20210A mutations.9 Kuhli-Hattenbach et al.,10 in a prospective study of 228 patients with RVO, aimed to assess the role of a hypercoagulable state (FII:G20210A mutation was not analysed), concluded that the presence of thrombophilic factors was more common in patients younger than 45 years and those without VFR or a personal history of thrombosis in another location. However, another prospective study11 found that the prevalence of inherited thrombophilia was not different between patients younger and older than 50 years. Besides, Glueck et al.12 found that high serum homocysteine levels and elevated anticardiolipin antibodies titers were more frequent in RVO patients than in controls (odds ratio [OR]; 8.6 and 6.3, respectively).

Taking into account the considerations mentioned earlier, the aim of our study was to analyse the prevalence of thrombophilic factors in a sample of patients with RVO, compared with a population-based group of age- and sex-matched controls. Additionally, we also analysed the prescribed treatment and the outcome of RVO patients.

Patients and methods
We conducted a prospective case–control study, between December 2008 and February 2014, at the University Hospital Marqués de Valdecilla, a tertiary-care center that serves as a reference hospital for a population of 350,000 inhabitants in Northern Spain. Based on clinical, fundoscopic and angiographic criteria, all patients diagnosed with RVO at the Department of Ophthalmology, were referred and assessed at our internal medicine department. All of them received an optimized therapy for high blood pressure, dyslipidemia and diabetes mellitus, according to the current guidelines. Those with serum hyperhomocysteinemia were treated with oral folic acid and vitamin B₁₂ supplementation.

All patients were followed-up until the VRF was controlled according to the current guidelines, and in those with APS, follow-up is ongoing to take into consideration anticoagulation if needed. The median (interquartile range [IQR]) follow-up period was 27 (15–49) months. A control group of 170 subjects taking a population-based group of age- and sex-matched controls. A control group of 170 subjects taking a population-based group of age- and sex-matched controls.

Laboratory parameters
Blood samples were obtained from an antecubital vein in the morning, after a requested 12-h overnight fast. Routine biochemical parameters were measured by standard automated methods in an ADVIA 2400 Chemistry System autoanalyzer (Siemens). Serum homocysteine was determined using a BN II nephelometer (Siemens) in 113 patients and was considered high if serum levels were >14.87 μmol/l. After June 2012, homocysteine was determined by chemiluminescence (Immulite, Siemens) in all the controls (n = 61) and 57 RVO patients, and was considered high if >15 μmol/l. Regarding coagulation studies, blood samples were collected in vacutainer tubes containing NaCitrate 3.2% in 1/9 proportion. After centrifugation (2500 rpm), 1 ml aliquots were stored at −30°C and tested within 38 days. The hypercoagulability study included platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, LA, anticardiolipin and anti-β₂ glycoprotein I antibodies, protein C, protein S and antithrombin level and FV:Q506 and FII:G20210A mutations. The following techniques were used: PT: a coagulometric method with Thromborel S (Siemens Healthcare Diagnostics) as a reagent; aPTT: a coagulometric method with Actin FSL reagent (Siemens); antithrombin: a chromogenic method with the Innova Antithrombin reagent (Siemens); protein C: a chromogenic method and Berichrom Protein C reagent (Siemens); free protein S: ELISA and DC-ELA PS Free reagent (Diagnostic Grifols) and activated protein C resistance (APC-R): a coagulometric method with Pefakit APC-R reagent (Pentapharm). LA was determined with the hexagonal phase phospholipid neutralization test by coagulometric method and Staclot LA reagent (Diagnostica Stago). dRVVT was tested using a coagulometric method and screening and confirmation reagent (Siemens). Serum anticardiolipin and anti-β₂ glycoprotein I antibodies were determined using ELISA and AESKULISA reagent (Diagnostics). The study of the FII:G20210A and FV:Q506 mutations was performed using polymerase chain reaction (PCR) analysis (Roche).

We used the CA-1500 autoanalyzer when Siemens reagents were analysed and the STA compact analyser (Diasys Stago) when using Stago and APC-R reagents. ELISA techniques were conducted with Triturus analyser (Diagnostic Grifols) and the PCR techniques with Light Cycler 2.0 analyser (Roche). Normal values were established according to 100 control patients of the same age range and gender. These values were as follows: antithrombin, 85–140%; protein C, 85–140%; protein S, 70–120%; LA, positive or negative; neutralizing aPTT < 10s; dRVVT, dyslipidemia (serum total cholesterol or triglyceride levels > 230 mg/dl and 150 mg/dl, respectively, in at least two repeated measurements after fasting for 12 h, or being on lipid-lowering drugs), diabetes mellitus (according to the American Diabetes Association [ADA] criteria),14 type of RVO (central or branches), first episode of RVO or recurrence, past or present history of thromboembolic disease in another location, onset of a new RVO during follow-up and prescribed treatments. We also recorded the presence of rheumatic diseases or malignancies. Patients with blood pressure below 140/90 mm Hg were analysed as a whole. Patients with high-normal blood pressure (a systolic pressure of 120–139 mm Hg and diastolic pressure of 80–89 mm Hg) were followed-up, initially with weekly control, then monthly, and in doubtful cases, we performed ambulatory blood pressure monitoring, to check that they did not fulfil the criteria to be considered as hypertensive subjects. None of the subjects were taking anovulatory drugs or receiving hormone replacement therapy.

Clinical variables
The variables analysed were as follows age, sex, high blood pressure (≥140/90 mm Hg or being on antihypertensive agents), dyslipidemia (serum total cholesterol or triglyceride levels > 230 mg/dl and 150 mg/dl, respectively, in at least two repeated measurements after fasting for 12 h, or being on lipid-lowering drugs), diabetes mellitus (according to the American Diabetes Association [ADA] criteria),14 type of RVO (central or branches), first episode of RVO or recurrence, past or present history of thromboembolic disease in another location, onset of a new RVO during follow-up and prescribed treatments. We also recorded the presence of rheumatic diseases or malignancies. Patients with blood pressure below 140/90 mm Hg were analysed as a whole. Patients with high-normal blood pressure (a systolic pressure of 120–139 mm Hg and diastolic pressure of 80–89 mm Hg) were followed-up, initially with weekly control, then monthly, and in doubtful cases, we performed ambulatory blood pressure monitoring, to check that they did not fulfil the criteria to be considered as hypertensive subjects. None of the subjects were taking anovulatory drugs or receiving hormone replacement therapy.
The hyper-lyses were conducted using SPSS 15.0 (Chicago, IL). The hypercoagulability study was repeated at least on one occasion in all the cases in which it was abnormal and, if found abnormal, confirmed with a third study. In patients or controls whose initial test was positive for LA or antiphospholipid antibodies (aPLs), but whose second test was negative, we perform a third test after 6 weeks and compute the results of this third test. In cases with positive LA and/or aPLs, antinuclear antibodies (ANAs) were determined by indirect immunofluorescence. A titer > 1/160 was considered positive.

For the sake of completeness, all patients underwent a 12-lead electrocardiogram to diagnose potentially embolic arrhythmias, such as atrial fibrillation.

Statistical analysis

Quantitative variables were expressed as mean ± Standard deviation (SD) and qualitative variables as percentages. Quantitative variables were compared using the Student t-test or Mann-Whitney U-test as appropriate and qualitative variables using the Fisher chi-squared test. OR and 95% CI (confidence interval) were used to compare cases and controls regarding coagulation parameters. A P value < 0.05 was considered statistically significant in all the calculations. All analyses were conducted using SPSS 15.0 (Chicago, IL).

Results

One hundred and seventy patients (93 men and 77 women) with a mean age of 68 ± 11 years (range, 40–89 years) and 170 controls (80 men and 90 women; mean age, 67 ± 10 years; range, 50–92 years) were included in the study.

Serum homocysteine

Overall, hyperhomocysteinemia was detected in 62 of 170 cases (36.5%). Before June 2012, serum homocysteine levels were high in 26 of 113 RVO patients (23%), and after June 2012 (using a chemiluminescence method), hyperhomocysteinemia was found in 36 of 57 cases (63.2%) and 15 of 61 (24.6%) controls (OR 5.2, 95% CI 2.7–10.1; \( P < 0.0001 \))

Acquired thrombophilia

Table 1 shows the distribution of positive LA and/or aPLs, in cases and controls.

Genetic thrombophilia

The following congenital disorders were observed in 21 (13%) patients with RVO: protein S deficiency (n = 8), antithrombin deficiency (n = 6), heterozygous FII:G20210A mutation (n = 3), heterozygous FV:Q506 mutation (n = 2), protein C deficiency (n = 2) and combined protein S and protein C deficiency (n = 1). Protein S deficiency was detected in two patients with chronic hepatitis C virus infection, one of them on interferon therapy.

Type of RVO and thromboembolic events other than retinal

Retinal impairment was of the branch type in 113 (66.5%) patients (110 temporal and 3 nasal) and central in 57 (33.5%), ratio 2:1. There were no significant differences with regard to age, thrombophilia or VRF between both types. RVO presented as a first episode in 164 cases (96.5%) and was recurrent in 6 (none of them had thrombophilia, but one patient was on acenocoumarol).

Patients with RVO had experienced a thromboembolic event outside the retinal veins. Two of them (40%) reported arterial thrombosis and were diagnosed with APS. There were no differences regarding coagulation parameters in the group of patients with previous thromboembolic events vs. those without but in APS was near to significance \( (P = 0.07) \). During the follow-up period, three patients had a new RVO, although none of them showed any abnormality in the coagulation tests.

Age subgroups

Eleven RVO patients were younger than 50 years, five of them (45.5%) had coagulation disorders: aPLs (n = 1), protein C deficiency (n = 2) and protein S deficiency (n = 2). Genetic thrombophilia was more prevalent in patients with RVO younger than 50 years compared with those aged more than 50 (36.4 vs. 11.6%; \( P = 0.04 \)).

VRFs subgroups

At baseline, serum low density lipoprotein (LDL) cholesterol levels were 128.9 ± 33.9 mg/dl in patients with RVO and 125.4 ± 30.8 mg/dl in controls \( (P = 0.32) \). The correspondent figures for high density lipoprotein (HDL) cholesterol were 56.7 ± 15.3 and 59.9 ± 15.3 mg/dl, respectively \( (P = 0.045) \). The percentage of cases and controls on statins, at the beginning of the study, was quite similar (~29%). Eighteen patients with RVO did not have classical VRF (high blood pressure, hypercholesterolemia or diabetes mellitus). Of them, the hypercoagulability study showed any abnormality in 9 (50%): heterozygous FV:Q506 mutation (n = 2), protein C deficiency (n = 2), protein S deficiency (n = 2), antithrombin deficiency (n = 1), heterozygous FII:G20210A mutation (n = 1) and combined aPLs and AL (n = 1). The prevalence of thrombophilia was significantly higher than in those with these VRF \( (P = 0.01) \).

Pharmacological therapy

Pharmacological therapy prescribed in RVO patients is shown in the flow chart (Figure 1). Anticoagulants were initiated in eight patients after the RVO diagnosis (acenocoumarol in all cases). This treatment was continued indefinitely in five of them, due to APS (n = 4) or protein C deficiency (n = 1). In the remainder patients, acenocoumarol was prescribed for 3 months, and then aspirin was started indefinitely. Low molecular weight heparin (LMWH) was prescribed to one patient during the first month. Thirteen individuals in the control group were taking acenocoumarol due to atrial fibrillation (n = 10) or a previous thromboembolic disease (n = 3).
Atrial fibrillation was only detected at RVO diagnosis in one case; anticoagulation was proposed, but the patient refused it, and then aspirin was initiated. None of our patients had systemic lupus erythematosus (in two patients with APS ANAs were positive), and one had rheumatoid arthritis. Active malignancies were found in two patients with RVO (colon and pancreas adenocarcinoma), but none of them showed coagulation disorders. Nine patients had a history of non-active neoplasms (diagnosed at least 3 years before RVO onset): prostate adenocarcinoma (n=5), breast carcinoma (n=1), renal carcinoma (n=1), non-Hodgkin lymphoma (n=1) and oligodendroglioma (n=1).

**Discussion**

In this study, we have showed that patients with RVO have a greater prevalence of LA/aPLs and hyperhomocysteinemia than controls. Furthermore, we have identified two well-defined subgroups of RVO patients with greater prevalence of genetic or acquired thrombophilic disorders: those without classical VRF and those younger than 50 years.

Unlike other thromboembolic diseases, in RVO the coagulation disorders mainly associated with an arterial involvement (hyperhomocysteinemia and positive LA/aPLs) predominate over venous (congenital thrombophilia). Thus, hyperhomocysteinemia is considered as an independent predictor for the development of atherosclerosis and thrombosis, and indeed, is a risk factor for RVO. In our series, hyperhomocysteinemia was found in 36.5% of patients. This figure is similar to other studies on RVO in which it varies between 34 and 49% but higher than 13% reported by Sartori et al. Noteworthy, hyperhomocysteinemia was five times higher in RVO patients than controls, using the same technique.

In patients with RVO, the prevalence of APS was 10%, and AL or aPLs, 6%, respectively. It was quite similar to Spanish and international series on patients with deep vein thrombosis. Our patients had a prevalence of APS, AL and aPLs similar to RVO published studies. Noteworthy, aPLs and AL were found in 2.8 and 1.8% of our controls, a figure slightly higher than 1.4%, and 0.8% reported in blood donors or in other series. Therefore, OR for these coagulation disorders were lower than reported in RVO patients, probably due to our controls were taken from a population-based cohort. In a largest meta-analysis that included 412 cases and 508 controls, the OR associated with anticardiolipin antibodies in RVO patients was 3.9 (95% CI: 2.3–6.7). The ratio was 6:1 for LA (5 case–control studies, 16 positive cases in 315 patients with RVO and 3 in 382 controls). Other investigators found similar results. Unlike Carbone et al., we did not find a higher frequency of ANA or systemic lupus erythematosus in our patients with RVO. Neither active malignancy nor rheumatic diseases, as a cause of acquired thrombophilia, were prevalent in our series.

Hereditary thrombophilia was detected in 13% of our patients with RVO. This figure was slightly greater than reported in controls from other series (10%), although was lower than 24–37% observed in patients with deep vein thrombosis.

Protein S deficiency (5%) and antithrombin deficiency (4%) were the most prevalent conditions we have found, also lower than 24–37% reported in patients with deep venous thrombosis. Janssen et al. reported a slight increase of prevalence of APC-R and FV:Q506 mutation, and Russo et al. found a slight increase of FII:G20210A mutation in RVO patients. However, Martinez et al. does not find any difference regarding genetic thrombophilia. According to these data, and unlike deep venous thrombosis, inherited thrombophilia seems to have no relevant role in the pathogenesis of RVO. Nevertheless, we have found that the presence of genetic thrombophilia was high in patients younger than 50 years. Besides, altered coagulation tests were observed in RVO patients who did not have VRF.

On the basis of these results and those previously published, although the benefit of a specific study of thrombophilia in RVO is not established, it seems reasonable to rule out APS in all patients presenting with this disorder. Moreover, as inherited thrombophilia has little influence on RVO, searching for a genetic thrombophilia seems advisable only in patients younger than 50 years and those who are older but have no classical VRFs.

The optimal treatment for RVO (antiplatelet, anticoagulation or fibrinolysis) has not yet been established. Squizzato et al. in a meta-analysis of 384 patients (234 with central retinal vein thrombosis and 150 with branch involvement), suggested that LMWH may be most effective, in terms of visual prognosis, in the acute phase of the disease. Lazo-Langner et al. have also suggested the superiority of LMWH compared with aspirin. Di Capua et al. showed high rate of vascular recurrence in patients with previous RVO, with a lower, although not significant, prevalence in those taking aspirin. Given that patients with RVO constitute a heterogeneous group, from an etiological standpoint (because in...
most cases atherosclerosis predominates, and to a lesser extent, thrombophilia), and are also different in terms of thrombosis progression time, it is difficult to establish a single treatment for all patients. Noteworthy, 7% of our patients were on oral anticoagulation due to previous atrial fibrillation, and this therapy was unable to prevent the development of RVO, despite maintaining an International Normalized Ratio (INR) within the therapeutic range. Moreover, RVO is associated with an increased risk of stroke in patients with atrial fibrillation. Therefore, RVO may be considered as a previous thromboembolic event when evaluating stroke risk in patients with this arrhythmia.

The main limitation of our study is that we did not perform genetic thrombophilia tests in our controls, due to the small number of RVO patients younger than 50. However, our study has some strengths. First, this is a prospective study in which we have included all the cases with RVO in our area. Second, our patients have a long-term follow-up. Third, we have compared, for the first time, our results with a well-defined population-based cohort from our region, therefore including subjects on anticoagulants.

In summary, in all patients with RVO, vascular risk should be adjusted toward the most optimal levels, correcting those factors susceptible to be treated, according to the current guidelines and promoting smoking cessation. Vitamin supplementation may also be taken into account by clinicians to correct hyperhomocysteinemia. In those patients in whom some type of thrombophilia is detected, prophylaxis with LMWH is indicated for situations at risk for venous thrombosis. Moreover, individualized assessment of anticoagulation therapy should be considered in patients with APS or congenital disorders with greater thrombotic potential. In the remaining patients, accounting for the vast majority of RVO cases, it is advisable, from a cardiovascular risk point of view, to prescribe antiplatelet agents, generally aspirin.

Conflict of interest: None declared.

References


