

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

The prevalence of activated protein C (APC) resistance and factor V Leiden is significantly higher in patients with retinal vein occlusion without general risk factors

Case-control study and meta-analysis

Matus Rehak^{1,2}, Jiri Rehak², Marc Müller³, Susanne Faude¹, Frank Faude¹, Annelie Siegemund⁴, Vera Krcova⁵, Ludek Slavik⁵, Dirk Hasenclever⁶, Markus Scholz⁶, Peter Wiedemann¹

¹Department of Ophthalmology, University Hospital Leipzig, Leipzig, Germany; ²Department of Ophthalmology, University Hospital Olomouc, Olomouc, Czech Republic; ³Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany; ⁴Intensive Care Unit, University Hospital Leipzig, Leipzig, Germany; ⁵Department of Haematology and Oncology, University Hospital Olomouc, Olomouc, Czech Republic; ⁶Institute for Medical Informatics, Statistics and Epidemiology (IMISE), University of Leipzig, Leipzig, Germany

Summary

Several small case-control studies have investigated whether factor V Leiden (FVL) is a risk factor for retinal vein occlusion (RVO) and generated conflicting data. To clarify this question we performed a large two-centre case-control study and a meta-analysis of published studies. Two hundred seven consecutive patients with RVO and a control group of 150 subjects were screened between 1996 and 2006. A systematic meta-analysis was done combining our study with further 17 published European case-control studies. APC resistance was detected in 16 out of 207 (7.7%) patients and eight out of 150 (5.3%) controls. The odds ratio (OR) estimated was 1.49 with a (non-significant) 95% confidence interval (CI) of 0.62–3.57. The meta-analysis including 18 studies with a total of 1,748 patients and 2,716 con-

trols showed a significantly higher prevalence of FVL in patients with RVO compared to healthy controls (combined OR 1.66; 95% CI 1.19–2.32). All single studies combined in the meta-analysis were too small to reliably detect the effect individually. This explains the seemingly contradictory data in the literature. In conclusion, the prevalence of APC resistance (and FVL) is increased in patients with RVO compared to controls, but the effect is only moderate. Therefore, there is no indication for general screening of factor V mutation in all patients with RVO. We recommend this test to be performed in patients older than 50 years with an additional history of thromboembolic event and in younger patients without general risk factors like hypertension.

Keywords

Retinal vein occlusion, factor V Leiden, APC resistance, activated protein C, meta-analysis

Thromb Haemost 2008; 99: 925–929

Introduction

Retinal vein occlusion (RVO) is the most common retinal vascular disease after diabetic retinopathy, involving the central trunks or branches of the retinal vein. Its exact pathogenesis is still unclear (1). Previous studies showed an increased risk of RVO in patients with hypertension, hypercholesterolemia and diabetes mellitus (2). Most recent studies have shown the role of hyperhomocysteinemia and antiphospholipid antibodies (3, 4). Furthermore, a platelet hyperaggregability might be an important

factor in the development of RVO (5), but prospective interventional trials are needed to assess the role of antiplatelet therapy (6, 7).

The literature on the association between thrombophilic factors and RVO consists only of small studies and case reports (8–24). The results of published investigations are inconclusive, and there is no agreement on which thrombophilic factor should be investigated in patients with RVO. One of the most discussed risk factors is the role of factor V Leiden (FVL).

Correspondence to:
Prof. Dr. med. Peter Wiedemann
Department of Ophthalmology, University Leipzig
Liebigstrasse 10–14, 04103 Leipzig, Germany
Tel.: +49 341 972 1650, Fax: +49 341 972 1659
E-mail: augen@medizin.uni-leipzig.de

Received November 4, 2007
Accepted after major revision March 24, 2008

Prepublished online April 9, 2008
doi:10.1160/TH07-11-0658

The aim of the present study was to examine the role of activated protein C (APC) resistance and/or FVL in a larger group of patients with RVO and to perform a meta-analysis of published European studies.

Patients and methods

Our study population consisted of 207 patients with RVO consecutively investigated at the Department of Ophthalmology, Palacky-University in Olomouc (Czech Republic) and the Department of Ophthalmology, University of Leipzig (Germany) between 1996 and 2006. Only patients with RVO less than three months after onset were included. The diagnosis of central retinal vein occlusion (CRVO) required the presence of retinal haemorrhage and veins with dilatation and tortuosity in all four quadrants. Branch retinal vein occlusion (BRVO) was defined as haemorrhage in the region of an occluded vein at an arteriovenous crossing. Exclusion criteria were ocular inflammatory diseases (Morbus Behcet and other vasculitis). Blood samples from all patients were obtained together with lifestyle information such as history of smoking, oral contraceptives, and other factors predisposing to thrombosis. Our control group consisted of patients with similar age and sex distribution seen for non-thrombotic eye diseases. Both cohorts were subdivided according to age in those younger or older than 50 years (< 50 and > 50 years). The study was approved by the appropriate Institutional Review Boards (University of Leipzig and Palacky University in Olomouc).

Blood was collected in vacutainer tubes containing 0.5 ml of 0.12 M sodium citrate and centrifuged at 2,000 g for 20 minutes. APC sensitivity ratios in patients and controls were determined by Coatest APC Resistance V system (Coatest® APC™ Resistance; Chromogenix, Mölndal, Sweden). The activated partial thromboplastin time (aPTT) was measured twice, once in the presence of a defined quantity of APC and in its absence, respectively. The quotient between the two clotting times was ex-

pressed as the APC ratio. Healthy individuals had an APC ratio in the range between 2–5. APC resistance was considered positive with repeated results of an APC ratio of less than 2. Since January 1998 the screening for APC resistance and the genetic analysis of the FVL was carried out by polymerase chain reaction (PCR) in all patients and controls. We used allele specific PCR with detection of products by electrophoresis on high-resolution gel (Technoclone, Austria).

Meta-analysis

Selection of studies: A MEDLINE literature search was performed to identify studies published up to January 2007 which examined the relationship between FVL (measured by PCR) or APC resistance (assessed by the modified APC resistance test with factor V-deficient plasma) and RVO. The following keywords and combinations of these words were used in the search: retinal vein occlusion, pathogenesis, haematological disorders, thrombophilia, FV Leiden and APC resistance. Additional articles and abstracts thought relevant were selected from review of the bibliographies of the articles generated from above search. In total, 147 articles were screened. Only studies with adequate control group reporting one of the above endpoints from European populations were included. To avoid acknowledged or covert duplication of data, only the most recent and complete series reported by investigators were included in the analysis. Our own data presented here were also included in the meta-analysis.

Statistical analyses

Odds ratios (OR) and respective 95% confidence intervals (CI) were calculated for each study and presented as a forest plot. OR greater than 1 imply increased prevalence of FVL mutation in patients with RVO. Standard random-effect meta-analysis was used to estimate a combined OR. The Q-statistic was used to assess heterogeneity. A funnel plot (not shown) was used to check for evidence of publication bias (25). Robustness of significant

Table 1: Characteristics of investigated patients and controls (n, %).

	All patients	Patients ≤50	Patients > 50	All controls	Controls ≤50	Controls > 50
Number (n)	207	50	157	150	47	103
Mean age (range) in years	65.5 (19 – 88)	44 (19 – 50)	72.5 (51 – 88)	61.3 (21 – 89)	43 (21 – 50)	69.5 (50 – 89)
Male (%)	93 (44.9%)	20 (40%)	73 (46.5%)	67 (44.6%)	20 (42.5%)	47 (45.6%)
Female (%)	114 (55%)	30 (60%)	84 (53.5%)	83 (55.3%)	27 (57.4%)	56 (54.3%)
CRVO	173 (83.6%)	41 (82%)	132 (84%)	-	-	-
BRVO	34 (16.4%)	9 (18%)	25 (16%)	-	-	-
APC resistance	16 (7.7%)	4 (8%)	12 (7.6%)	8 (5.3%)	4 (8.5%)	4 (3.8%)
Smokers	29 (14%)	14 (28%)	15 (9.5%)	14 (9.3%)	4 (8.5%)	10 (9.7%)
Hypertension	96 (46.3%)	19 (38%)	77 (49%)	23 (15.3%)	5 (10.6%)	18 (17.5%)
Diabetes	26 (12.6%)	3 (6%)	23 (14.6%)	5 (3.3%)	1 (2.1%)	4 (3.9%)
Oral contraceptives (% of females)	4 (3.5%)	3 (10%)	1 (1.2%)	2 (2.4%)	2 (4.3%)	-
History of thrombosis	7 (3.4%)	4 (8%)	3 (2%)	2 (1.3%)	-	2 (1.95%)

Table 2: Prevalence of risk factors analysed in patients subdivided into to two age subgroups.

	All patients APC resistance Positive	All patients APC resistance negative	Patients ≤50 APC resistance positive	Patients ≤50 APC resistance negative	Patients > 50 APC resistance positive	Patients > 50 APC resistance negative
Number (n)	16	191	4	46	12	145
Smokers	2 (12.5%)	27 (14.1%)	1 (25%)	13 (28%)	1 (8.3%)	14(9.6%)
Hypertension	2 (12.5%)	94 (49.2%)	0	19 (41%)	2 (17%)	75(52%)
Diabetes	2 (12.5%)	24 (12.5%)	0	3 (6.5%)	2 (17%)	21(14.5%)
Oral contraceptives (% of females)	1 (6.3%)	3 (2.1%)	1 (25%)	2 (4.3%)	0	1(0.7%)
History of thrombosis	4 (25%)	3 (1.6%)	2 (50%)	2 (4.3%)	2 (17%)	1(0.7%)

results was investigated by a jackknife procedure. If significance was lost by leaving out single studies, we interpreted the result as insufficient evidence for an effect. Calculations were performed using the “meta” package of the statistical software “R” (www.r-project.org) (26).

For our patients, an analysis of general risk factors was performed. Prevalence of these factors was compared between the group of patients positive for APC resistance and patients without APC resistance using Fisher’s exact test. The analysis was also done for age-stratified subgroups. We considered a p-value of less than 0.05 as statistically significant.

Results

Characteristics of patients and controls of our own study are detailed in Table 1.

APC resistance was seen in 16 of the 207 patients (7.7%) and eight of the 150 controls (5.3%) (OR 1.49, 95%CI 0.62–3.57). FV Leiden was found in six of the 76 patients (7.9%) enrolled since 1998 and investigated for APC resistance and FVL. No differences between results of APC resistance and FVL analysis were found in this group of patients. In the subgroup of patients younger than 50 years, four of the 50 patients (8%) and four of 47 controls (8.5%) were positive for APC resistance (OR 0.93, 95%CI 0.22–3.97). In subjects older than 50 years, 12 out of 157 patients (7.6%) and four out of 103 controls (3.8%) APC resistance could be observed (OR 2.05, 95%CI 0.64–6.53). None of these results were significant.

The analysis of risk factors (Table 2) comparing patients positive for APC resistance with APC-negative patients showed significant differences in prevalence of hypertension and history of thrombosis. Hypertension was found in 12.5% of the patients with APC resistance and in 49.2% of the patients without APC resistance ($p=0.0072$). This effect could be observed also in the subgroup of patients older than 50 years ($p=0.032$). The difference in the group of younger patients was not significant. The prevalence of thromboembolic events in patients with APC resistance (both age groups) was significantly higher than in patients without APC resistance (elder patients $p=0.015$, younger patients $p=0.028$). For other risk factors no statistically significant differences were found.

Results of 17 European case-control studies on the role of FVL in patients with RVO and the result of the present study are summarised in Table 3. There was significant heterogeneity in the data (see Forest plot Figure 1), mainly due to two studies. The study of Weger et al. (2) included only patients with BRVO while the study of Greiner et al. (13) reported an unusually high prevalence of FVL mutation. No significant heterogeneity ($p=0.57$) would be present without these studies. Using a random effects model, the estimate of the combined OR for the prevalence of FVL (APC resistance) in RVO compared with controls was 1.66 (95% CI 1.19–2.32, $p=0.0026$). Significance of results is pre-

Table 3: Summary of studies included in the meta-analysis with number of patients and odds ratios (OR) in particular studies.

Nr.	Study, Year of publication	Patients/controls	Odds ratio
1.	Linna [18], 1997	46/142	2.11
2.	Larsson [20], 1997	83/101	1.00
3.	Albisinni [10], 1998	36/68	8.38
4.	Demirci [15], 1999	50/120	0.86
5.	Greiner [13], 1999	81/581	3.74
6.	Kalayci [17], 1999	52/81	1.04
7.	Larsson [12], 1999	37/288	1.87
8.	Marcucci [11], 2001	100/100	3.27
9.	Aras [21], 2001	40/50	0.82
10.	Bombelli [22], 2002	68/120	0.65
11.	Kuhli [19], 2002	142/128	2.05
12.	Marcucci [23], 2003	55/61	5.02
13.	Cruciani [16], 2003	29/62	0.69
14.	Gori [24], 2004	112/112	2.81
15.	Weger [2], 2005	294/294	0.72
16.	Arsene [9], 2005	234/180	1.16
17.	Gumus [8], 2006	82/78	2.69
18.	Rehak – present study	207/150	1.49
	Combined	1748/2716	1.66

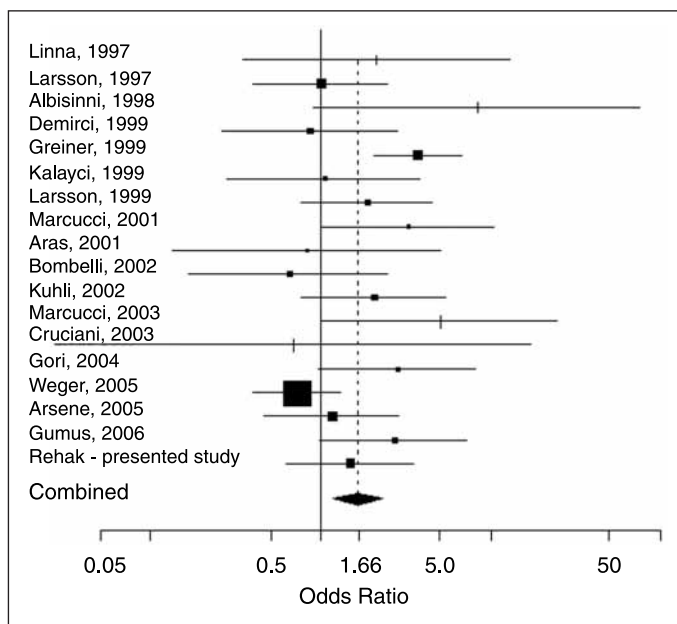


Figure 1: Funnel plot of studies included in the meta-analysis.

served after dropping single studies, as well as the two studies mentioned above (2, 13). Dropping the study of Weger et al. (2) would slightly increase the effect to 1.87 (95% CI 1.39–2.53, $p < 0.0001$); dropping the study of Greiner et al. (13) would slightly decrease the effect to OR 1.47 (95% CI 1.09–1.98, $p = 0.012$). No indication for publication bias was found (Egger's test, Funnel plot).

Discussion

Several risk factors have been proposed for the development of RVO. Abnormalities of the coagulation system leading to thrombophilia have been studied, but their role remains controversial.

APC resistance, mainly caused by FVL mutation, has been shown to be an independent risk factor for the development of deep vein thrombosis (28, 29). However, the association between this risk factor and RVO is still unclear. Several smaller studies with contradictory results have been published so far. The presented study of 207 patients failed to demonstrate any significant difference in the prevalence of APC resistance between patients with RVO (7.7%) and the prevalence in the group of controls (5.3%). The observed prevalence in controls is consistent with published prevalence rates of FVL in the Caucasian population. In 95% of cases with APC resistance, the cause is a single point mutation in the factor V gene (R506Q), the so called FVL mutation (27).

First generation assays for APC resistance were limited by false positive findings in patients with high factor VIII:C levels or lupus anticoagulants antibodies. A modified APC resistance test, in which the plasma samples are diluted in factor V-deficient plasma, is very specific for APC resistance caused by FVL (30, 31). This assay was used in all investigated patients. Additionally, FVL determination by PCR was used in the patients included since 1998. Owing to the high sensitivity and specificity

of the modified APC resistance test, comparison of our results with the results of other studies which investigated FVL using the PCR technique is justified.

Given the known differences in prevalence of FVL in different ethnic groups, we restricted our analysis to European populations to achieve homogeneity in regard to genetics and lifestyle. We analyzed 17 European studies published up to January 2007 and our own data, including in total 1,748 patients and 2,716 controls. As result of the meta-analysis, we found a significant higher probability in the presence of FVL in cases than in controls (OR 1.66). Additionally, we found roughly the same size of effect in our own study population, though our sample size alone was not sufficient to achieve significance. Significant heterogeneity between studies was observed. However, dropping two conspicuous studies (2, 13) changed effect size only slightly and did not abrogated significance of the effect.

We performed a sample size calculation for a single study to confirm the effect of higher prevalence of FVL in RVO. Assuming the effect size observed in the meta-analysis, approximately 1,500 patients and controls would be required to achieve a power of 95%. All single studies combined in the meta-analysis were too small to reliably detect the effect individually. Thus the small sample size of published studies explains at least partially the inconsistency of published results about the role of the FVL in patients with RVO.

The results of our meta-analysis are in line with a report from Janssen et al. (4). However, their study included only 792 patients / 1,418 controls, and the required size of about 1,500 cases was not achieved. Our meta-analysis includes 1,748 patients / 2,716 controls and therefore has higher statistical power.

As in younger patients other risk factors (hypertension, advance atherosclerosis, diabetes mellitus) are rare, one may hypothesise that the attributed risk and prevalence of FVL is more important in these patients. It seems to be likely that an inherited thrombotic risk factor, when present, should manifest in younger age (9, 19). The definition of younger and elderly individuals varies considerably in literature with a cut-off ranging between 45 and 60 years.

In our study 8% of patients younger than 50 years and 7.6% of patients older than 50 years showed APC resistance. This is in line with other reports (14, 16, 18). Because only three studies (9, 18, 19) analyzed the prevalence of FVL in different age groups, it was not possible to study the influence of age on the prevalence in the meta-analysis.

However, the study reports provided only incomplete information comparing characteristics regarding the presence of known risk factors in patients and controls. It was therefore not possible to assess the quality of the selection of the control group systematically.

As mentioned above, the pathogenesis of RVO is multifactorial. The general risk factors (hypertension, diabetes, and smoking) seem to play the most important role. In our study, the prevalence of hypertension was significantly higher in the group of elderly patients (> 50 years) negative for APC resistance. In younger patients the difference was not statistically significant, probably due to a smaller size of the groups (4 positive and 46 negative for APC resistance). In both age subgroups of patients with APC resistance the prevalence of history of thromboem-

bolic events was significantly higher than in patients negative for APC resistance. The analysis of risk factors confirmed the hypothesis that hypertension plays an important role in the pathogenesis of RVO. Patients without hypertension as well as patients with positive history of thromboembolic events have a significantly higher probability to be positive for APC resistance. Therefore, the role of APC resistance should be evaluated in combination with evaluation of these risk factors.

The role of family history of thrombosis in the management of patients with RVO is not clear. Only few studies investigated prevalence of thrombosis in RVO patients' relatives. No significant differences in family history of deep vein thrombosis in patients with RVO compared to controls were found (20). Currently, no prospective randomized study demonstrated the benefit concerning visual prognosis in patients with RVO treated with anticoagulation. On the other hand, no study investigated the effect of anticoagulation separately in RVO patients with FVL.

In conclusion, combining our own study with the data published in the literature, we showed that the prevalence of FVL is higher in patients with RVO compared to controls. However the effect is only small. We recommend to ask for history of thromboembolic events and to check the general risk factors in all patients with RVO. Screening of FVL is not necessary in patients older than 50 years with general risk factors or with a negative history of thrombosis. We recommend to investigate the FVL in RVO patients younger than 50 years and in RVO patients without general risk factors or with a positive history of thrombotic events.

Abbreviations

RVO, retinal vein occlusion; CRVO, central retinal vein occlusion; BRVO, branch retinal vein occlusion; APC, activated protein C; FV Leiden, factor V Leiden mutation; OR, odds ratio; CI, confidence intervals.

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