

## Activated protein C resistance in patients with central retinal vein occlusion

J Larsson, A Sellman, B Bauer

### Abstract

**Aim/background**—A new defect in the anticoagulant system has recently been discovered—activated protein C resistance. The frequency of this disorder has been shown to be increased in young patients (<50 years of age) with central retinal vein occlusion. This study was carried out to determine if there was any overrepresentation of activated protein C resistance in patients >50 years of age with central retinal vein occlusion.

**Methods**—Blood samples were obtained from 83 patients >50 years of age and with a history of central retinal vein occlusion. The blood samples were analysed for activated protein C resistance with standard clinical laboratory methods.

**Results**—In this material 11% of the patients were resistant to activated protein C. The normal incidence of activated protein C resistance in the same geographical area is 10–11%.

**Conclusion**—Activated protein C resistance does not seem to be a cause of central retinal vein occlusion in people older than 50 years.

(*Br J Ophthalmol* 1997;81:832–834)

Central retinal vein occlusion (CRVO) is a disease that is fairly common in elderly people. Glaucoma, hypertension, atherosclerosis, and diabetes are factors that are well known to be associated with the disease.<sup>1</sup> Hereditary deficiencies in the coagulation system have never been proved to be an important factor in the aetiology of CRVO. In patients with a diagnosis of thrombophilia, hereditary deficiencies in the coagulation system have been found only in 4–6%.<sup>2,3</sup>

In 1992 Dahlbäck *et al* discovered that some young patients with thrombosis had a resistance to activated protein C.<sup>4,5</sup> Although activated protein C (APC) was added to the patients' plasma it did not result in the normal inhibition of the coagulation. Later they were able to show that this was due to a selective defect in the anticoagulant function of factor Va. The defect is a point mutation that has been localised to the locus for the factor V gene

on chromosome 1-fV<sub>506</sub>,<sup>6–8</sup> and it is dominantly inherited.<sup>9</sup>

The prevalence of this mutation varies between 2% and 15% depending on the geographical area.<sup>7,10–12</sup> It is absent in African, Japanese, and Chinese people<sup>13,14</sup> and has one of its peak figures in southern Sweden where it reaches 10–15% in the population.<sup>15</sup> The risk of thrombosis is increased five to tenfold in heterozygotes and 50–100-fold in homozygotes.<sup>16–18</sup> Several patients with a history of thrombosis, mainly in the leg, have been examined with regard to APC resistance and the prevalence has been found to vary between 21% to 52%, with the higher values if there is a history of thrombophilia in the family.<sup>7,9,19,20</sup>

We have recently shown that activated protein C resistance was increased fourfold in a group of young patients with CRVO, aged less than 50.<sup>21</sup> We wanted to find out whether patients above that age also had an overrepresentation of APC resistance and the fV<sub>506</sub> mutation.

### Patients and methods

#### PATIENTS

The population sample was recruited from the patient records of the eye clinics of the Lund University Hospital and the Helsingborg Medical Center Hospital in Helsingborg, Sweden. Of the 108 patients found, blood samples were obtainable from 83. They comprised 45 (54%) males and 38 (46%) females. The average age was 70 years (range 50–92 years).

The control patients collected by Holm *et al*<sup>15</sup> from the University Hospital in Malmö were used since they belong to the same geographical area. All the patients were from the same geographical area, which could be defined as a circle with a diameter no more than 80 km. This is important since we know that the frequency of APC resistance varies with even fairly small geographical distances.

#### METHODS

The analysis of resistance to APC was carried out at the Department of Clinical Chemistry, Malmö, Sweden. The method is the same as that described by Dahlbäck *et al*.<sup>22</sup> Seventy five of the patients were also tested with the modified APC resistance test in which the plasma samples were diluted 1/5 in factor V deficient

Department of  
Ophthalmology, Lund  
University Hospital,  
Sweden

J Larsson  
B Bauer

Medical Center  
Hospital of  
Helsingborg, Sweden  
A Sellman

Correspondence to:  
Dr J Larsson, Department of  
Ophthalmology, Lund  
University Hospital, S-221  
85 Lund, Sweden.

Accepted for publication  
26 June 1997

plasma. This modified test gives a better discrimination between normal and APC resistant patients.<sup>23-25</sup> The eight patients who were tested with the standard method only were included in the study before the modified test was available.

The analysis of the fV<sub>506</sub> mutation was carried out at the Department of Clinical Chemistry, Malmö, Sweden. The method is the same as that described by Zöller *et al.*<sup>26</sup>

Patients were considered as APC resistant when their plasma samples had an APC ratio less than 2.4 in the standard test and 1.9 in the modified test. All patients that showed APC resistance in the test were screened for the fV<sub>506</sub> mutation. The patients who had borderline APC ratios—that is, <2.2 in the modified test, were also screened for the fV<sub>506</sub> mutation.

### Results

Nine (11%) patients were APC resistant and they did all have the fV<sub>506</sub> mutation. Of these nine, eight were heterozygotes and one was homozygous for the mutation. The average APC ratio in the modified test for these patients was 1.5 (range 1.1–1.6). The average APC ratio in the modified test in the remaining patients was 2.3 (range 2.5–2.2).

The eight patients who were tested only with the standard method all had normal APC ratios and were not borderline cases. They had an average APC ratio of 3.7 (range 3.1–4.8).

Four patients had an APC ratio of 2.1 in the modified test and were thus screened for the fV<sub>506</sub> mutation, but none of them had the mutation.

The control group is the same as that used by Holm *et al.*,<sup>15</sup> and consisted of 101 healthy volunteers with no history of thrombosis. In the control group 11 (11%) were APC resistant. Thus there was no difference in APC resistance between the controls and the patients with CRVO; the 95% confidence interval for the difference in APC resistance prevalence between the patient and the control group was 0% (SD 9%)

We also compared the clinical picture between the patients with APC resistance and the normal ones, but there was no distinguishable difference between them.

### Discussion

This study of 83 patients shows that APC resistance is not more common in patients more than 50 years of age with central retinal vein occlusion than in the normal population. Williamson *et al* found a twofold increase in the frequency of APC resistance in 56 patients with CRVO (average age 67.5 years).<sup>27</sup> They did not screen for the fV<sub>506</sub> mutation in those patients who showed APC resistance, which is probably because the fV<sub>506</sub> mutation analysis was not available at the time they did their investigation. An explanation of the difference between our and their results could be that it is well known that the APC resistance test sometimes gives discrepancies when you test the same blood sample twice,<sup>20 28-30</sup> and this makes it important to verify your test result with an analysis of the fV<sub>506</sub> mutation. Freyburger *et al*

looked at APC resistance in 130 patients (mean age 62 years) with retinal vein occlusion—that is, branch, hemi, and central retinal vein occlusion together, and they used four different batches for the APC resistance test.<sup>31</sup> They found that 11% of the patients and 6% of the controls were APC resistant, but there was a variability between the batches and when screening 12 of the patients with APC resistance none of them carried the fV<sub>506</sub> mutation. Thus, it is most important to confirm the APC resistance with an analysis of the fV<sub>506</sub> mutation. In normal clinical practice it has been shown that more than 90% of the patients with a positive APC resistance test have the fV<sub>506</sub> mutation.<sup>7 8 26 30 32</sup>

Earlier we showed that APC resistance was increased fourfold in a group of patients younger than 50 years,<sup>21</sup> so it seems to be an important factor in the aetiology of CRVO in young patients. However, when that study was performed the mutation test was not yet available, and so we did not examine the prevalence of the fV<sub>506</sub> mutation; the results from that study have yet to be confirmed. Recently Linna *et al* were not able to show an excess of the factor fV<sub>506</sub> mutation in a group of 46 patients younger than 50 years and with a diagnosis of CRVO or branch retinal vein occlusion (BRVO).<sup>33</sup> The discrepancy between their results and ours in this younger group of patients could be due to the fact that we only included patients with CRVO, whereas they examined those with BRVO and CRVO.

Since the most important risk factors for central retinal vein occlusion—glaucoma, hypertension, and atherosclerosis—are factors that are much more common in the elderly, it is probable that these factors have such an impact on the aetiology to CRVO that APC resistance is of less importance. On the other hand, in younger people glaucoma, hypertension, and atherosclerosis are much less common, and a deficiency in the coagulation system is more likely to be the triggering factor for thrombosis.

In conclusion, we have shown that activated protein C resistance does not seem to be an important factor in the aetiology of CRVO in patient older than 50 years.

This study was supported by the Carmen and Bertil Régners Foundation for Ophthalmological Research and the Elsa and Ola Ohlssons Family Foundation for Ophthalmological Research, Sweden.

We would also like to thank Harald Andersson at the Institution for Oncology, Lund University Hospital, Sweden, for help with the statistical analysis.

- 1 McGrath MA, Wechsler F, Hunyor ABL, Penny R. Systemic factors contributory to retinal vein occlusion. *Arch Intern Med* 1978;138:216–20.
- 2 Malm J, Laurell M, Nilsson IM, Dahlbäck B. Thromboembolic disease—critical evaluation of laboratory investigation: see comments. *Thromb Haemost* 1992;68:7–13.
- 3 Taberno MD, Tomas JF, Alberca I, Orfao A, Borrascas AL, Vicente V. Incidence and clinical characteristics of hereditary disorders associated with venous thrombosis. *Am J Hematol* 1991;36:249–54.
- 4 Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci USA* 1993;90:1004–8.
- 5 Dahlbäck B, Svensson PJ. Nyupptäckt rubbning i antikoagulationen resistens mot protein C orsak till trombos. *Lakartidningen* 1994;91:50–3.

- 6 Dahlbäck B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. *Haemostasis* 1994;24:139-51.
- 7 Bertina RM, Koelman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;369:64-7.
- 8 Greengard JS, Xiao Xiu XS, Fernandez JA, Griffin JH, Evatt B. Activated protein C resistance caused by Arg506Gln mutation in factor Va. *Lancet* 1994;343:1361-2.
- 9 Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med* 1994;330:517-22.
- 10 Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med* 1995;332:912-7.
- 11 van Bockxmeer FM, Baker RI, Taylor RR. Premature ischaemic heart disease and the gene for coagulation factor V (letter). *Nature Med* 1995;1:185.
- 12 Hellgren M, Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thromboembolism associated with pregnancy and oral contraceptives. *Am J Obstet Gynecol* 1995;173:210-3.
- 13 Chan LC, Bourke C, Lam CK, Liu AW, Brookes S, Jenkins V, et al. Lack of activated protein C resistance in healthy Hong Kong Chinese blood donors—correlation with absence of Arg506-Gln mutation of factor V gene (letter). *Thromb Haemost* 1996;75:522-3.
- 14 Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *Lancet* 1995;346:1133-4.
- 15 Holm J, Zoller B, Berntorp E, Erhardt L, Dahlbäck B. Prevalence of factor V gene mutation among myocardial infarction patients and healthy controls is higher in Sweden than in other countries. *J Intern Med* 1996;239:221-6.
- 16 Majerus PW. Human genetics. Bad blood by mutation (news; comment). *Nature* 1994;369:14-5.
- 17 Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 1995;85:1504-8.
- 18 Dahlbäck B, Hillarp A, Rosen S, Zoller B. Resistance to activated protein C, the FV:Q506 allele, and venous thrombosis. *Ann Hematol* 1996;72:166-76.
- 19 Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden thrombophilia study. *Lancet* 1993;342:1503-6.
- 20 Legnani C, Palareti G, Biagi R, Coccheri S. Activated protein C resistance in deep vein thrombosis. *Lancet* 1994;343:541-2.
- 21 Larsson J, Olafsdottir E, Bauer B. Activated protein C resistance in young adults with central retinal vein occlusion (see comments). *Br J Ophthalmol* 1996;80:200-2.
- 22 Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci USA* 1993;90:1004-8.
- 23 Denson KW, Reed SV, Haddon ME. The modified APC resistance test (letter). *Thromb Haemost* 1995;74:995.
- 24 Trossaert M, Conard J, Horellou MH, Elalamy I, Samama MM. The modified APC resistance test in the presence of factor V deficient plasma can be used in patients without oral anticoagulant (letter). *Thromb Haemost* 1996;75:521-2.
- 25 Jorquera JL, Montoro JM, Fernandez MA, Aznar JA, Aznar J. Modified test for activated protein C resistance (letter) (see comments). *Lancet* 1994;344:1162-3.
- 26 Zoller B, Svensson PJ, He X, Dahlbäck B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. *J Clin Invest* 1994;94:2521-4.
- 27 Williamson TH, Rumley A, Lowe GD. Blood viscosity, coagulation, and activated protein C resistance in central retinal vein occlusion: a population controlled study (see comments). *Br J Ophthalmol* 1996;80:203-8.
- 28 Alhenc Gelos M, Gandrille S, Aubry ML, Emmerich J, Flessinger JN, Aiach M. Unexplained thrombosis and factor V Leiden mutation (letter) (see comments). *Lancet* 1994;344:555-6.
- 29 Baker R, Thom J, van Bockxmeer F. Diagnosis of activated protein C resistance (factor V Leiden) (letter; comment). *Lancet* 1994;344:1162.
- 30 Voorberg J, Roelse J, Koopman R, Buller H, Berends F, ten Cate JW, et al. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V (see comments). *Lancet* 1994;343:1535-6.
- 31 Freyburger G, Bilhou-Nabera C, Dief S, Javorschi S, Labrousse S, Lerebeller MJ, et al. Technical and biological conditions influencing the functional APC resistance test. *Thromb Haemost* 1996;75:460-5.
- 32 Zoller B, Dahlbäck B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis (see comments). *Lancet* 1994;343:1536-8.
- 33 Linna T, Ylikorkkala A, Kontula K, Puska P, Tervo T. Prevalence of factor V Leiden in young adults with retinal vein occlusion. *Thromb Haemost* 1997;77:212-6.