Blood viscosity, coagulation, and activated protein C resistance in central retinal vein occlusion: a population controlled study

Tom H Williamson, Ann Rumley, Gordon D O Lowe

Abstract
Background—The role of blood viscosity and haemostasis has been investigated in mixed groups of patients with branch and central retinal vein occlusion (CRVO) with conflicting results. This may have partly been due to the different aetiologies of these two types of vein occlusion.

Methods—In this study viscosity and coagulation (including activated protein C resistance) were examined in 87 patients with CRVO and compared with the results from an age-matched, population based control group.

Results—Viscosity variables were higher in CRVO than in controls which suggested that reduced red cell deformability was associated with the occurrence of CRVO. A higher percentage of the patients with CRVO (12%) had activated protein C resistance than controls (5%). Patients who developed the complication of iris neovascularisation had relatively low antithrombin III, factor VII, and tissue plasminogen activator indicating both a tendency to thrombus formation and a reduction in fibrinolytic activity.

Conclusion—Increased blood viscosity may contribute to the production of CRVO by inducing stasis of blood flow, with thrombus formation in at risk individuals who go on to develop iris neovascularisation.

(Br J Ophthalmol 1996; 80: 203–208)

Blood viscosity is an important determinant of blood flow and may be a contributory factor in the production of a number of vascular diseases of the eye. A clinical association between central retinal vein occlusion (CRVO) and raised blood viscosity exists because the systemic hyperviscosity syndromes often present with retinal features which are similar to or indistinguishable from CRVO.10 Furthermore, the retinopathy only occurs when the blood viscosity is high11 and resolves when therapy reduces the viscosity.1213 Another link with CRVO and viscosity has been provided by the association between the systemic conditions found in CRVO— for example, systemic hypertension, and abnormal rheology.14

There is evidence that CRVO and branch vein occlusion have different aetiologies—for example, a raised erythrocyte sedimentation rate (a measure of increased red cell aggregation) is common in patients with CRVO but not in those with branch retinal vein occlusion.15 For this reason, the two conditions should not be combined in studies looking at aetiological factors, and yet almost all studies of viscosity have included mixed groups.16–21 These have described raised haematocrit, haematocrit corrected whole blood viscosity and plasma viscosity during16 and after the acute stage,19 and higher whole blood viscosity in ischaemic vein occlusions than non-ischaemic. Elevated fibrinogen levels16 or red cell aggregation22 may be the cause of the raised viscosity. Other studies have, however, found similar rheology results in patients and controls.2021 A comparison of CRVO with a population based control group has not been performed and would elucidate the rheology characteristics in the condition. Furthermore, little is currently known about the natural history of the viscosity changes; therefore, it would be useful to examine serial blood samples in these patients.

Various haemostatic factors have been implicated in retinal vein occlusion—for example, increased factor VIII which has a coagulant activity, reduced antithrombin III which is an inhibitor of blood coagulation, and increased fibrinopeptide A which indicates the activation of blood coagulation.1923 Recently, low activated protein C resistance has been described in patients with thrombotic tendencies.24 Inheritance of this deficiency is thought to be considerably more common in the general population (3–6%) than deficiencies of proteins C, S, and antithrombin III. Although a case of activated protein C resistance and CRVO has been described, no cohort studies of the prevalence of this abnormality in CRVO have been performed.25

In this study, only patients with CRVO (or its variant, hemiretinal vein occlusion) were examined, thereby removing the influence of patients with branch vein occlusions on the results. The patients were age matched to individuals attending population surveys, thereby reducing bias from employing hospital based controls. For the first time activated protein C resistance was investigated in a group of patients with CRVO.2427 Finally, the natural history of raised viscosity was studied by serial measurements over a year after onset of the condition.

Patients and methods
Eighty seven patients with CRVO were examined prospectively (including 10 patients with hemiretinal vein occlusion which was considered as a variant of CRVO). The patients
were compared with a control group taken from a local population study of rheology.\textsuperscript{26} The results were compared in a case controlled manner with each case matched for year of age. This provided 69 patients and 69 controls for comparison.

Unfortunately, owing to commencing examination of haemostasis at a later stage in the study the haemostatic variables were not available for all of the patients. Results from 62 patients were analysed. Forty seven patients were age matched to 47 controls from another local population study for comparison (Lowe \textit{et al}, unpublished data). Thirty five patients were age matched to 35 controls for analysis of activated protein C. In a similar fashion 25 patients were age matched to 25 controls for comparison of protein C and S. Age matching in a case controlled manner was unavailable from the population based study for von Willebrand’s factor, tissue plasminogen activator, and plasminogen activator inhibitor. The results from the patients over the age of 64 years were compared with the results from a local population study of individuals above this age. Twenty five patients with an ischaemic grade of retinopathy were age matched to 25 with non-ischaemic CRVO. The protocol employed in the ischaemic grading is provided in Table 1.

The patients were examined again at least 1 year after the onset of their occlusion for the development of complications. In this part of the analysis only the results from patients initially examined less than 3 months from onset, were analysed. The results from nine patients who went on to develop iris neovascularisation were compared with the results from 22 patients who did not develop this complication. Three patients who presented with neovascularisation and one patient who developed retinal neovascularisation were excluded.

### BLOOD SAMPLING

Fifteen ml of venous blood was sampled from an antecubital vein through a 21 gauge butterfly needle after minimal use of a tourniquet. The blood sampling was performed between 11 am and 12 noon in all patients. Five ml of blood were anticoagulated with dipotassium edetate (K$_2$EDTA, 1·5 mg/ml) for viscosity examination which was performed within 4 hours of sampling. Nine ml of blood were placed directly into a tube with 1 ml trisodium citrate (0·109 M) and, within 1 hour of the sampling, centrifuged at 15 000 rpm (25 000 g) for 10 minutes. The plasma was then divided into seven separate sample tubes and stored at −30°C for 48 hours. The samples were then transported in ice to a −70°C freezer. In a group of 20 patients examined within 1 month of onset of the occlusion, the rheology samples were repeated at 6 months and at 1 year. Blood rheology was measured as previously described.\textsuperscript{28}

### HAEMOSTATIC VARIABLES

Fibrinogen was measured by the Clauss method on a Coag-A-mate X2 coagulometer using the manufacturer’s reagents and international standard (Organon Teknika, Cambridge). Clotting factors VII, VIII, and IX were recorded by one stage clotting methods on an ACL300 coagulometer similarly employing the manufacturer’s reagents and international standards (Instrumentation Laboratories, Warrington). Antithrombin III was measured by the manufacturer’s chromogenic assay in serum or plasma. Plasminogen activator inhibitor activity was measured chromogenically (Quadratrec, Epsom, Surrey) and tissue plasminogen activator antigen measured by an enzyme linked immunosorbent assay (ELISA) technique (Biopool, Stockholm, Sweden). von Willebrand factor antigen was recorded using an ELISA technique (Dako Ltd, High Wycombe, Bucks). Activated protein C resistance (Coastex, Chromogenix, Stockholm, Sweden) was also measured (ACL 300R, Instrumentation laboratories, Warrington)\textsuperscript{24} 27 and resistance defined as a value less than 2·1.\textsuperscript{27

### STATISTICAL ANALYSIS

The results were compared by two tailed Student’s t test, Spearman rank correlation, or analysis of covariance (with the covariant the age of the patient) where appropriate: 95% confidence limits are provided.

#### Results

### BLOOD VISCOSITY - WHOLE GROUP (TABLE 2)

There were a total of 44 males and 43 females with a mean age of 67·5 years (range 27–87 years). The mean duration from onset of the retinal vein occlusion was 5 months (range 1–12 months).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood viscosity (mPa·s)</td>
<td>3·41</td>
<td>0·6</td>
</tr>
<tr>
<td>Plasma viscosity (mPa·s)</td>
<td>3·43</td>
<td>0·43</td>
</tr>
<tr>
<td>Corrected blood viscosity (mPa·s)</td>
<td>3·43</td>
<td>0·43</td>
</tr>
<tr>
<td>Relative blood viscosity</td>
<td>2·56</td>
<td>0·26</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>43·3</td>
<td>4·7</td>
</tr>
<tr>
<td>Red cell aggregation (arbitrary units)</td>
<td>4·22</td>
<td>1·29</td>
</tr>
</tbody>
</table>

*Values missing for some patients.

### Table 1. The criteria for the classification of central retinal vein occlusion as ischaemic

<table>
<thead>
<tr>
<th>Ischaemic:</th>
<th>AND</th>
<th>Non-ischaemic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Fluorescein angiographic evidence of severe capillary dropout (&gt;10 disc diameters) or if the fluorescein is not assessable</td>
<td>(1) No capillary drop out on fluorescein angiography</td>
<td>(1) Partially ischaemic: Any case which cannot be fitted into the above categories</td>
</tr>
<tr>
<td>(2) Presence of iris neovascularisation OR</td>
<td>(2) Absence of iris neovascularisation</td>
<td></td>
</tr>
<tr>
<td>(3) At least two of the following</td>
<td>(3) Normal ERG</td>
<td></td>
</tr>
<tr>
<td>(a) Reduction in the B/A wave amplitude of the ERG</td>
<td>(a) RAPD of at least 20 degrees (cross polarisers)</td>
<td></td>
</tr>
<tr>
<td>(b) RAPD of at least 20 degrees</td>
<td>(b)</td>
<td></td>
</tr>
<tr>
<td>(c) Visual acuity less than 6/60</td>
<td>(c)</td>
<td></td>
</tr>
</tbody>
</table>

RAPD=relative afferent pupillary defect.
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Table 4 The significantly different haemostatic variables between central retinal vein occlusion (CRVO) and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Confidence limits</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin III (iu/dl)</td>
<td>101·8</td>
<td>13·0</td>
<td>107·0–98·9</td>
<td>0·02</td>
</tr>
<tr>
<td>Controls</td>
<td>95·1</td>
<td>11·5</td>
<td>99·1–102·5</td>
<td></td>
</tr>
<tr>
<td>von Willebrand factor (iu/dl)</td>
<td>141</td>
<td>56</td>
<td>157–126</td>
<td>0·05</td>
</tr>
<tr>
<td>CRVO</td>
<td>126</td>
<td>49</td>
<td>134–119</td>
<td></td>
</tr>
<tr>
<td>Plasminogen activator inhibitor (% pool)</td>
<td>35</td>
<td>107–84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>33</td>
<td>83–68</td>
<td></td>
<td>0·0001</td>
</tr>
<tr>
<td>Activated protein C resistance</td>
<td>2·62</td>
<td>0·5</td>
<td>2·78–2·46</td>
<td>0·05</td>
</tr>
<tr>
<td>CRVO</td>
<td>2·85</td>
<td>0·5</td>
<td>3·01–2·69</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>3·01</td>
<td>2·69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean plasma viscosity was lower in the ischaemic group than in the non-ischaemic patients. Mean protein S was significantly higher in the non-ischaemic group. No difference was found in any of the other variables.

Table 5 The significantly different variables for those patients with retinal ischaemia and those who developed iris neovascularisation (NVI)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Confidence limits</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal ischaemia:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viscosity (mPa·s)</td>
<td>1·31</td>
<td>0·07</td>
<td>1·34–1·28</td>
<td>0·03</td>
</tr>
<tr>
<td>Ischaemic</td>
<td>1·38</td>
<td>0·11</td>
<td>1·43–1·33</td>
<td></td>
</tr>
<tr>
<td>Non-ischaemic</td>
<td>1·23</td>
<td>0·15</td>
<td>1·35–1·12</td>
<td></td>
</tr>
<tr>
<td>Protein S</td>
<td>1·40</td>
<td>0·22</td>
<td>1·51–1·29</td>
<td></td>
</tr>
<tr>
<td>Ischaemic</td>
<td>1·38</td>
<td>0·11</td>
<td>1·43–1·33</td>
<td></td>
</tr>
<tr>
<td>Non-ischaemic</td>
<td>1·23</td>
<td>0·15</td>
<td>1·35–1·12</td>
<td></td>
</tr>
<tr>
<td>Development of iris neovascularisation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VII (iu/dl)</td>
<td>95·7</td>
<td>16·3</td>
<td>114–77</td>
<td>0·05</td>
</tr>
<tr>
<td>No NVI</td>
<td>135·8</td>
<td>20·8</td>
<td>136–116</td>
<td></td>
</tr>
<tr>
<td>Antithrombin III (iu/dl)</td>
<td>92·8</td>
<td>12·1</td>
<td>109–76</td>
<td>0·04</td>
</tr>
<tr>
<td>No NVI</td>
<td>107·4</td>
<td>9·9</td>
<td>113–102</td>
<td></td>
</tr>
<tr>
<td>Tissue plasminogen activator (ng/ml)</td>
<td>4·62</td>
<td>2·0</td>
<td>7·5–17</td>
<td></td>
</tr>
<tr>
<td>No NVI</td>
<td>7·25</td>
<td>2·5</td>
<td>8·5–6·0</td>
<td>0·03</td>
</tr>
</tbody>
</table>

The mean corrected and relative blood viscosities were higher in patients with CRVO than in the controls (Figs 1 and 2). Random blood cholesterol was positively correlated with the plasma viscosity (r=0·36, p=0·001). Haematocrit and, therefore, whole blood viscosity were significantly higher (p=0·01 and 0·007 respectively) in patients who smoked cigarettes than in those who did not.

Haemostatic variables – whole group (Table 3)

The results for all of the patients with CRVO are shown in Table 3 with significant results from the case controlled analysis shown in Table 4. Mean antithrombin III, von Willebrand factor, and plasminogen activator inhibitor were higher in patients than in controls. Mean activated protein C was lower in patients than in controls and a higher proportion of patients had activated protein C resistance (12·5% compared with 5% in controls). The clinical results of patients with CRVO and activated protein C resistance were examined but no particular pattern of presentation was distinguishable.

Retinal ischaemia (Table 5)

The mean plasma viscosity was lower in the ischaemic group than in the non-ischaemic patients. Mean protein S was significantly higher in the non-ischaemic group. No difference was found in any of the other variables.

Development of iris neovascularisation (Table 5)

Patients who were examined in the acute stage of the condition and who went on to develop iris neovascularisation had significantly lower factor VII, antithrombin III, and tissue plasminogen activator compared with those who did not develop neovascular complications. There were no differences in blood viscosity variables.
blood viscosity, haematocrit, corrected blood viscosity, and relative blood viscosity were all lower at 1 year after occlusion than at the onset (all, p<0.05, Figs 3 and 4). Haematocrit corrected blood viscosity was also lower at 1 year than 6 months (p<0.05).

Discussion

BLOOD VISCOSITY

In this study, both haematocrit corrected and relative blood viscosity were higher in patients with CRVO than in the age-matched control population, but no significant elevation was detected in erythrocyte aggregation or fibrinogen. The raised relative blood viscosity suggests that cellular factors— that is, cellular deformability, caused the increase in viscosity. In explanation, firstly, haematocrit corrected blood viscosity was calculated to remove the contribution of haematocrit on blood viscosity and, secondly, relative blood viscosity was calculated to negate the influence of plasma viscosity. Relative blood viscosity, therefore, provides an indirect measure of the viscosity resulting from cellular factors in the blood such as deformability and aggregation. As there was no difference in red cell aggregation between the two groups, blood cell deformability was likely to be the main cause of the increased viscosity in these patients.

Higher viscosity was found at the time of onset of the occlusion than 1 year after the onset. The occlusion of the central retinal vein may, therefore, occur at a time of raised viscosity for some, as yet undetermined, reason. Alternatively, general medical advice given to the patient at presentation—for example, on reducing blood pressure or lipid levels, may have resulted in a reduction in viscosity.

Significantly higher whole blood viscosities in the ischaemic form of retinal vein occlusion (in studies including branch retinal vein occlusion) than in the non-ischaemic variant have been described previously. Indeed, stagnation of blood flow in the microcirculation secondary to the elevated viscosity has been used as an explanation for the capillary non-perfusion seen on fluorescein angiography. In this study, however, viscosity was not related to the development of retinal ischaemia or iris neovascularisation. Indeed, a relatively lower plasma viscosity was found in the patients with ischaemic features.

HAEMOSTASIS

The production of a blood clot first involves aggregation of platelets upon a site of damaged vascular endothelium, followed by the stabilisation and extension of this aggregate by fibrin, which is converted from the soluble protein fibrinogen via the intrinsic and extrinsic cascade reactions of various clotting factors. This thrombotic mechanism is balanced by factors which prevent formation of the clot—for example, antithrombin III, protein C, protein S, and by the fibrinolytic system which converts plasminogen to plasmin which in turn acts to lyse fibrin and fibrinogen. The fibrinolytic system involves the interaction of factors such as tissue plasminogen activator (increasing fibrinolysis) and plasminogen activator inhibitor (reducing fibrinolysis). In some circumstances an imbalance of these systems helps to cause thrombosis.

The role of increased coagulation activity and reduced fibrinolysis in CRVO is as yet unknown. In this study, although significant differences were found, care must be taken in the interpretation as confidence limits on the results were wide in many cases. Histological studies suggest that thrombus formation occurs in the retinal vein, therefore haemostatic systems may be important. However, most of the cases that have been reported histologically have had rubeotic glaucoma and the few that have been examined in the acute stage of the disorder without rubeosis have all presented atypically. In this study, an increase in the antithrombin III level in patients with CRVO was observed, in contradiction to the deficiency of this factor in a previous study where perhaps antithrombin III levels were reduced because of the inclusion of patients with branch vein occlusion. It has been suggested that an increase in antithrombin III may sometimes occur, possibly as a compensatory
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reaction to increased coagulation factors because both low and high antithrombin III levels are associated with ischaemic heart disease. Increased von Willebrand factor in the patients with CRVO also indicated a relative tendency to thrombus formation and increased plasminogen activator inhibitor may have led to a relative reduction in fibrinolysis in these patients.

In this study 12.5% of the patients with CRVO had activated protein C resistance compared with 5% of the controls. This new measure is much commoner than other inherited thrombotic tendencies and may, therefore, be an important cause of vasculopathies such as CRVO. In contrast with the other haemostatic factors, which only demonstrated relative differences between groups of patients, activated protein C resistance was abnormal in a significant proportion of patients and might be worth routine clinical investigation. However, no particular pattern of clinical presentation of these patients was discernible that might help identify the patients with this problem.

Those patients who subsequently developed iris neovascularisation had lower levels of antithrombin III than patients who did not develop this complication, signifying an increased tendency to the formation of thrombus. A reduction in fibrinolytic activity was also evident from lower levels of tissue plasminogen activator (in the presence of unchanged plasminogen activator inhibitor), indicating a relative inability to lyse thrombus. Defective fibrinolysis has been associated before with new vessel formation. The patients with ruberosis iridis also had lower levels of coagulation factor VII and a trend to lower factor IX, changes which may partly balance the prothrombotic effects of low antithrombin III and tissue plasminogen activator. Although the patients classified as ischaemic had lower protein S (an anticoagulant non-enzymatic cofactor for protein C) no difference was detected in those patients who went on to develop ruberosis.

PATHOGENETIC MECHANISM FOR CRVO

The results suggest that increased viscosity produces a risk of CRVO and that abnormalities of haemostasis (by increasing the chance of thrombus formation) are particularly associated with the development of iris neovascularisation. Whether these findings are primarily responsible for the condition or merely associated, because conditions such as systemic hypertension and hyperlipidaemia are common in CRVO, remains unclear. Ring et al have previously suggested that a 'rheological obstruction' may occur in patients with retinal vein occlusion. The results imply that viscosity produces stasis of blood in the vein and explain why reduction of blood viscosity (isoviscosal haemodilution29 or troxerutin30) in the treatment of CRVO leads in some patients to a recovery of vision. It may be that thrombus does not occur in all patients, thus explaining why anticoagulation has been unsuccessful in treating CRVO.35 Stasis of blood flow in some 'at risk' individuals may result in the development of thrombosis within the vein, further reducing blood flow. The thrombus may persist in patients with a deficient fibrinolytic system, chronically reducing their retinal blood flow and thereby resulting in the development of neovascular complications. Indeed such a severe reduction of blood flow has been detected by colour Doppler imaging in patients with a risk of iris neovascularisation.36 37 The risk of this complication has been reduced by the use of streptokinase to break down thrombus38 but unfortunately this agent has also produced vitreous haemorrhage in some patients and has had little overall effect upon visual recovery.

In summary, our results suggest that raised blood viscosity (from reduced cell deformability) is present in patients with CRVO and that, in particular, those patients who develop neovascular complications have abnormalities of haemostasis. A significant proportion of the patients had activated protein C resistance, therefore it may be clinically appropriate to investigate patients with CRVO for this.

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