Hemostatic risk factors in ischemic stroke

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Summary
The role played by hemostasis in the pathogenesis of ischemic stroke is still controversial. In the present study, we looked for a possible association of ischemic stroke and high clotting activity of factor II (FII:C), factor V (FV:C), factor VII (FVII:C), factor X (FX:C) and fibrinogen. We investigated 157 non-anticoagulated patients (86 males, 71 females; median age 41 y; range 16-73), who had survived ischemic stroke for at least 2 months, and 193 healthy controls with similar age and sex distribution (104 males, 89 females; median age 39 y, range 19-74). Patients showed significantly higher body mass index, as well as significantly higher prevalence of arterial hypertension, smoking and hyperlipidemia. FV:C (p = 0.05), FX:C (p = 0.04) and fibrinogen (p = 0.05) were higher in patients as compared to controls. In a univariate risk analysis FX:C and FV:C were associated with the relative risk for ischemic stroke showing an odds ratio (OR) of up to 2.8 (95% CI: 1.05-7.6) and 3.4 (95%CI: 1.4-7.9), respectively, for levels above 130%. In a multivariate analysis using a logistic regression model including age, sex, arterial hypertension, smoking habit, diabetes, hyperlipidemia, BMI and the coagulation factors, FV:C was still found to significantly (p=0.03) add to the risk of ischemic stroke. An increase of factor FV:C by 10% was associated with an increase in the relative risk of 19% (95% CI.: 2%-38%). In conclusion, we found a high plasma level of FV:C to be a prevalent (FV:C > 130% in 20/157 patients) and independent risk factor for ischemic stroke.

Keywords
Ischemic stroke, risk factors, coagulation, factor V

Introduction
Stroke, both ischemic and hemorrhagic, is a major cause of death and disability in the industrialized countries (1, 2). Mostly stroke occurs in patients with one or more risk factors such as age (2), hypertension (3), smoking (4), diabetes mellitus (5), hyperlipidemia (6) or obesity (7). However, there is a significant proportion, particularly of young individuals, who suffer from ischemic stroke in the absence of these risk factors. Therefore, many epidemiologic studies have looked at other hereditary and acquired risk factors, especially in the field of hemostasis. As a result of these studies, several procoagulatory as well as anticoagulatory factors are discussed today as risk factors for ischemic stroke (for review see 8). The most important procoagulatory and fibrinolytic factors, respectively, to be mentioned in this context are fibrinogen, factor VII:C, factor VIII:C, von Willebrand factor, tPA antigen and fibrin D-dimers (9-16). Concerning the hereditary thrombophilias currently accepted as risk factors for venous thromboembolism, mainly factor V Leiden mutation, prothrombin G20210A transition and protein S deficiency are – however controversially – taken into account as possible risk factors for arterial disease, including ischemic stroke, especially in young individuals and children (17-25). Moreover, several studies have suggested an association with hyperhomocysteinemia (26, 27), and very recently protein Z has been linked with ischemic stroke (28, 29). Finally, antiphospholipid antibodies and lupus anticoagulant have been suggested to play a pathogenetic role in ischemic stroke.

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(30, 31). However, for all these factors and defects mentioned above, the results are still quite controversial. In the present study we investigated, in a case control design, the possible association of several anticoagulatory and procoagulatory factors with ischemic stroke, with special focus on the clotting activity of the coagulation factors II, V, VII, X and fibrinogen.

**Methods**

**Patients and controls**

The study cohort was selected from the 236 consecutive patients (132 male (m), 104 female (f), median age 39.9 years (y), range 1.4 - 77.1 y) referred to our department for thrombophilia investigation between 1988 and 1996 because of a history of ischemic stroke. Diagnosis of stroke was based on clinical assessment and on neuroimaging, either computerized tomography or magnetic resonance imaging. Patients were further investigated by neurovascular ultrasound or selective cerebral arteriography, transcranial and mostly transesophageal echocardiography, electrocardiography and, if indicated, by long-term electrocardiography. The neurologist classified patients according to the TOAST classification (32). Only patients with stroke of undetermined etiology (so-called cryptogenic stroke) were referred for thrombophilia investigation. For this study, patients with the following criteria were excluded: Age < 15 y (n = 18), interval between the stroke and thrombophilia investigation < 2 months (n = 19), time since withdrawal of oral anticoagulation < 1 month (n = 2), ongoing oral anticoagulation (n = 4), only clinical diagnosis of stroke (n = 26), stroke possibly related to a known cause such as essential thrombocythemia (n = 2), polycythemia vera (n = 2), cerebral irradiation (n = 2), vasculitis (n = 2), stenosis of carotid artery (n = 1) and cerebral angioma (n = 1). There remained 157 study patients (86 men, 71 women, median age 41 y, range 16 – 73 y) having suffered one or more objectively diagnosed cryptogenic strokes and being investigated 2 to 149 months (median 4.5 months) after the last cerebrovascular event. Sixteen of the 157 patients had suffered additional venous thromboembolism and 9 additional arterial thromboembolic events in other localizations. In 15 patients a patent foramen ovale was diagnosed. In none of them was venous thrombosis diagnosed, neither in association with the stroke nor had it been at any other time.

The control group was selected from students, physicians and laboratory staff, respectively. It consisted of 193 healthy volunteers with age (median 39 y, range 19 - 74 y) and gender (104 m, 89 f) similar to the patient group. Volunteers did not have a history of thromboembolic events or bleeding tendency, and were not taking oral contraceptives or postmenopausal hormone replacement therapy.

Arterial hypertension, diabetes mellitus as well as hyperlipidemia were diagnosed according to the history and/or medication of the patients and controls, respectively. Height and weight were measured or asked for calculation of body mass index (BMI in kg/m$^2$).

Patients and controls consented to the scientific analysis of their data. The clinical data of our study and control groups are presented in table 1.

**Blood sampling**

After obtaining 5 ml of EDTA-blood and 5 ml of native blood, 2 x 9 ml were collected into two plastic syringes (Monovette®, Sarstedt, Nümbrecht, Germany), each containing 1ml 0.106 M trisodium citrate. After twice centrifuging at 1500 $\times$ g for 10 minutes each at 15-18°C plasma was directly used for analysis or stored in small aliquots in polypropylene tubes at $-70^\circ$C.

**Assays**

The clotting activity of factor II (F II:C), factor V (F V:C), factor VII (F VII:C) and factor X (F X:C) was measured on a Fibrintimer (Dade Behring) by a prothrombin time based assay using Thromborel-S® (Dade Behring, Marburg, Germany) and the respective deficient substrate plasmas (Dade Behring). Activity was expressed as percentage of a normal human plasma pool (NHP) defined to contain 100% of clotting activity. Fibrinogen was determined according to the method of Clauss (33).

Antithrombin III (AT III) heparin cofactor activity, protein C (PC) anticoagulant and chromogenic activity after Protac$^\circ$ activation as well as free and total protein S (PS) antigen were measured using commercial kits.

Resistance to activated protein C (APC) was assayed using COATEST$^\circ$ APC$^TM$ Resistance V (Chromogenix, Mölndal, Sweden). Factor V R506Q was diagnosed according to our APC sensitivity ratio in-house cutoffs (normal $\geq$ 2.2, heterozygous $\geq$ 1.3 and < 1.9, and homozygous $\leq$ 1.15).

**Statistics**

Median and range or proportions for baseline risk factors were calculated for patients and controls. Comparison of continuous variables between patients and controls was performed by Mann-Whitney-U-test; categorical data were analyzed by chi-square test or Fisher’s exact test where appropriate. Correlation between observations was sought using Spearman’s rank correlation, expressed as the correlation coefficient and tested for significance using Spearman’s test for correlation. P-values were two-sided and were considered significant if $\leq$ 0.05. Correction for multiple comparisons was done, where appropriate, using the Bonferroni method (34). Confidence intervals (CI) were calculated at the 95% level. Statistical analysis was performed using Sigmastat (Jandell, Erkrath, Germany).

We calculated crude odds ratios (OR) by standard methodology as estimates of the relative risk for ischemic stroke for various strata of the coagulation factors II, V, VII and X. To
simultaneously adjust for the effects of other ischemic stroke risk factors, we used multiple logistic regression analysis (SAS System, SAS Institute Inc., Cary, N.C.). Adjustment was made for the dichotomized risk factors sex, arterial hypertension (yes/no), hyperlipidemia (yes/no), diabetes mellitus (yes/no), smoking (yes/no) and for the continuous variables age, BMI, fibrinogen and F II:C, F V:C, F VII:C and F X:C.

### Results

#### Baseline characteristics

The baseline characteristics of patients and controls are given in Table 1. There was no difference between the groups regarding age and sex. Diabetes mellitus was rare in both groups and did not differ significantly. However, patients showed significantly higher BMI (p = 0.003) as well as significantly higher prevalence of arterial hypertension (p = 0.02), current or former smoking (p = 0.01) and hyperlipidemia (p = 0.005), respectively.

#### Hereditary thrombophilic defects

Prevalence of deficiency of antithrombin, protein C and total protein S, respectively, as well as prevalence of APC resistance did not differ between patients and controls (data not shown). Concerning free protein S, female patients (2/71 tested patients) and controls (1/89 tested controls) did not show different prevalence of deficiency whereas male patients (10/86 tested patients) were significantly (p = 0.007) more frequently deficient than controls (2/104 tested controls).

#### Clotting factors

Comparison of FII:C, FV:C, FVII:C, FX:C and fibrinogen, respectively, in patients and controls showed significantly higher values for FV:C (p=0.05), FX:C (p=0.04) and fibrinogen (p=0.05), respectively, in the patient group (Table 2). No difference was found for FII:C and FVII:C. To further elucidate this observation, we subsequently looked for possible confounders by evaluating the correlation of the investigated coagulation factors with various clinical and laboratory findings. We found significant positive correlations of FII:C, FV:C, FVII:C, FX:C and fibrinogen, respectively, with age both in the patient and in the control groups (Table 3). Similarly, coagulation factors correlated with BMI, however, FII:C and FV:C did not reach statistical significance in the patient group (Table 3). Furthermore, we looked for possible associations with hypertension, hyperlipidemia and smoking habit, respectively, in the patients (Table 4). Compared to normotensive patients, patients with arterial hypertension showed higher activities of all these factors, FII:C being the only one not reaching statistical significance. In the patient group with hyperlipidemia the trend for higher values in comparison to patients with normal lipids was significant for FII:C and FX:C, and among smokers higher FII:C, FV:C and fibrinogen were noticed as compared to non-smokers. Considering the small number of hypertensive and hyperlipidemic controls, we restricted the correlation analysis in the control group to the smoking habit and found fibrinogen as the only factor being significantly higher in smokers as compared to non-smokers (data not shown). Correlation of the coagulation factors with each other was significantly positive in patients as well as in controls for all factors except for FVII:C with fibrinogen in patients (Table 5).
Next, we performed a univariate relative risk analysis for each coagulation factor to investigate whether high clotting activities were associated with an increased relative risk for ischemic stroke. Crude odds ratios (OR) were calculated for the three upper quartiles as compared to the lowest quartile. The only factor showing an elevated, however, not statistically significant OR was FV:C with an OR of 1.6 (95% CI: 0.9–2.9) when comparing the fourth quartile with the first quartile (data not shown). In addition, the risk analysis was performed using different cut-off levels (Fig. 1). We found an increasing relative risk for ischemic stroke with increasing FV:C levels with an odds ratio of up to 3.4 (95% CI: 1.4-7.9) for FV:C levels above 130%. FX:C levels above 130% were associated with an odds ratio of 2.8 (95% CI: 1.05-7.6) while high FII:C, FVII:C and fibrinogen plasma levels, respectively, did not prove to be significant risk factors. High FV:C and FX:C levels were prevalent findings with 20 (13%) out of 157 patients having FV:C > 130%, and 13 (8.2%) out of 157 patients presenting with FX:C > 130%.

Next, we performed a multivariate risk analysis using a logistic regression model in order to adjust for possible confounders including age, gender, diabetes mellitus, hyperlipidemia, arterial hypertension, smoking habit, BMI and the coagulation factors (Table 6). FV:C still contributed significantly (p=0.03) to the risk of ischemic stroke. An increase of FV:C by 10% of NHP was associated with an increase in the relative risk of 19% (95% CI.: 2-38%). FX:C, however, did no longer contribute significantly to the risk in the multivariate analysis.
Table 6: Multivariate risk analysis using a logistic regression model including various parameters*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Risk*</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>p-values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10 years</td>
<td>1.27</td>
<td>1.00 – 1.61</td>
<td>0.05</td>
</tr>
<tr>
<td>Gender</td>
<td>male</td>
<td>1.02</td>
<td>0.59 – 1.75</td>
<td>0.96</td>
</tr>
<tr>
<td>Hypertension</td>
<td>yes</td>
<td>1.41</td>
<td>0.55 – 3.60</td>
<td>0.47</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>yes</td>
<td>2.69</td>
<td>1.18 – 6.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes</td>
<td>yes</td>
<td>1.18</td>
<td>0.09 – 15.44</td>
<td>0.90</td>
</tr>
<tr>
<td>smoking</td>
<td>yes</td>
<td>1.35</td>
<td>0.77 – 2.35</td>
<td>0.30</td>
</tr>
<tr>
<td>BMI</td>
<td>5 Kg/m²</td>
<td>1.58</td>
<td>0.98 – 2.53</td>
<td>0.06</td>
</tr>
<tr>
<td>FII:C</td>
<td>10%</td>
<td>0.78</td>
<td>0.61 – 1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>FV:C</td>
<td>10%</td>
<td>1.19</td>
<td>1.02 – 1.38</td>
<td>0.03</td>
</tr>
<tr>
<td>FVII:C</td>
<td>10%</td>
<td>0.88</td>
<td>0.74 – 1.10</td>
<td>0.15</td>
</tr>
<tr>
<td>FX:C</td>
<td>10%</td>
<td>1.15</td>
<td>0.93 – 1.42</td>
<td>0.19</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>1 g/l</td>
<td>1.50</td>
<td>0.80 – 2.80</td>
<td>0.21</td>
</tr>
</tbody>
</table>

* Adjustment was made for the dichotomized risk factors gender, arterial hypertension (yes/no), hyperlipidemia (yes/no), diabetes mellitus (yes/no), smoking (yes/no) and for the continuous variables age, BMI, fibrinogen and FII:C, FV:C, FVII:C and FX:C.

Risk associated with the presence or increase of the respective parameter.

While FII:C was not a risk factor in the univariate analysis, multivariate analysis suggested an increase of FII:C to be protective, which is difficult to explain and needs further study.

**Discussion**

In the present study we compared 157 consecutive non-anticoagulated patients older than 15 years, who had survived objectively diagnosed ischemic stroke of undetermined etiology for ≥ 2 months, and had been referred to our institution for thrombophilia investigation with 193 healthy volunteers. The two groups were similar concerning gender and age. The patient group contained 1.2 times more men than women which is in accordance with the slight preponderance of men among stroke patients reported in the literature (7). The median age of 41 years (range 16-73) of the patients in this study was substantially lower than the typical age of a stroke population. This is due to a referral bias. Only patients in whom the etiology of the stroke was not obvious were referred for thrombophilia investigation, and this was evidently more common in younger individuals. We confirmed the established clinical risk factors, arterial hypertension, hyperlipidemia, smoking as well as high BMI, all being significantly more frequent in the study cohort than among controls.

The equal prevalence of deficiency of antithrombin and protein C, respectively, in patients and controls further suggests, together with the majority of reports in the literature, that these two hereditary defects do not seem to play a role in the pathogenesis of stroke. In contrast, isolated deficiency of free protein S (so-called protein S deficiency type III) was significantly more prevalent in male patients than in male controls. This observation can not be explained by the acute state of stroke, since the interval between stroke and our investigation was at least two months (median 4.5 months, range 2-149) in all cases, nor by vitamin K deficiency, which was excluded by the finding of normal values for FII:C, FVII:C and FX:C, respectively. Together with Factor V Leiden mutation, protein S deficiency belongs to the hereditary thrombophilias most frequently suggested by several authors to be associated with stroke (24).

However, the reports are controversial (35) and it should be stressed that we have not proven the hereditary nature of free protein S deficiency in our patients. Regarding the prevalence of factor V Leiden mutation, having been tested in our patients and controls by the modified APC resistance test showing an accepted sensitivity and specificity of almost 100%, we found no difference between the two groups, which is in accordance with the findings of Ridker et al. (22).

The main focus of our study was the investigation of a possible pathogenetic role of the clotting activities of the procoagulatory factors II (FII:C), V (FV:C), VII (FVII:C), X (FX:C) and fibrinogen. In the literature we find several reports stressing fibrinogen as a risk marker of stroke. Our patients showed slightly higher values for several of the above mentioned procoagulatory factors as compared to controls, the difference being significant for FV:C (p = 0.05), FX:C (p = 0.04) and fibrinogen (p = 0.05). Performing a univariate risk analysis for each coagulation factor by calculating crude ORs for the three upper quartiles of the clotting activities as compared with the lowest quartile, the only factor showing an elevated, however, statistically not significant OR of 1.6 (95% CI: 0.9-2.9) was FV:C. However, when different cut-off levels were applied, FV:C and FX:C proved to be significant risk factors with clotting activities above 130% showing an OR of 3.4 (95% CI: 1.4-7.9) for FV:C and 2.8 (95% CI: 1.0-7.6) for FX:C, respectively. High FV:C was prevalent in the patients, 13% showed a level > 130%. We found positive correlations of the coagulation factors with age (FII:C, V:C, VII:C, X:C, fibrinogen), BMI (FVII:C, X:C, fibrinogen), arterial hypertension (FV:C, VII:C, X:C, fibrinogen), hyperlipidemia (FII:C, X:C) and smoking (FII:C, V:C, fibrinogen). Therefore, in order to adjust for these possible confounders, we performed a multivariate risk analysis using a logistic regression model. In this analysis, FV:C was still found to significantly (p=0.03) add to the risk of ischemic stroke. An increase of FV:C by 10% of NHP raised the relative risk of stroke by 19% (95% CI: 2-38%). The other factors, however, did not contribute to the risk in the multivariate model.

In conclusion, this is the first report suggesting high FV:C to be a prevalent and independent risk factor for ischemic stroke. Recently, our group has shown a similar association with myocardial infarction (36). Considering the lack of similar reports in the field of venous thromboembolism, one may speculate that factor V might play a specific role in the pathogenesis of arterial thromboembolism. We are aware of the fact that we have investigated a highly selected stroke collective. The next steps will be to examine our observation of cryptogenic stroke.
patients in a more general stroke population and to elucidate the pathogenetic mechanism by which high F V:C contributes to arterial thromboembolic risk.

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References