intron-exon junctions were sequenced. No other mutation was identified. BspHI RFLP analysis confirmed homozygosity for this mutation in the proband and heterozygosity in her four siblings available for analysis (Figure 1C, Table 1). No such mutation was identified in the 165 control individuals.

Although in the absence of functional studies one cannot formally exclude that this amino acid change is a rare polymorphism, several observations indicate that the mutation we found is responsible for the patient’s factor XI deficiency. First, this was the only change noticed after having sequenced the complete F11 coding sequence and intron-exon junctions. Second, the mutation occurs in a highly conserved portion of the FXI serine protease catalytic domain. Indeed, comparisons of sequences from human (SwissProt accession number P03951), mouse (SwissProt accession number Q91Y47), rabbit (Genbank accession number AF395821), rat (Genbank accession number XM224872) and cow (TrEMBL accession number P79131), show conservation of the Thr575 residue. Although Thr and Val residues can be found in this position in the catalytic domains of other serine proteases, no Met has been reported. Other mutations in the active site have previously been shown to cause factor XI deficiency, including a mutation in the immediately adjacent residue: Ser576Arg. Third we found that controls did not have the mutation. Finally, the proband’s elder sister with normal values of factor XI did not have the mutation.

Interestingly the Thr575Met mutation was identified earlier this year in compound heterozygosity associated with a much more severe early truncating mutation (Q88X). No functional analysis or population screening for the Thr575Met was performed in this study. Our finding of the same mutation is additional evidence that it is indeed responsible for the defect. To date the majority of mutations identified in the factor XI gene seem to prevent or greatly reduce protein expression. In the present case the factor XI antigen level was 105 U/dL, and this therefore represents a dysfunctional CRM+ variant. A discrepancy between antigen level and activity level was also observed for the French patient heterozygous for Thr575Met (FXI:Ag 42 U/dL, FXI:Ac 455%).

Myrna Germanos-Haddad, Philippe de Moerloose, François Boehlen, Flora Peyvandi,* Margarette Neerman-Arbez**

*Hematology and Immunology Laboratory, Hôtel-Dieu Hospital, Beyrouth, Lebanon; **Division of Angiology and Hemostasis, University Hospital, Geneva, Switzerland; †Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, IRCCS Maggiore Hospital and University of Milan, Italy; *Department of Genetic Medicine and Development, University Medical School, Geneva, Switzerland

Funding: this study was supported by a grant from the Laboratoire Français du Fractionnement et des Biotechnologies (LFB).

Key words: factor XI deficiency, mutation, hemostasis, bleeding disorder.

Correspondence: Philippe de Moerloose, Unité d’Hémostase, University Hospital of Geneva, 1241 Geneva 14, Switzerland. Phone: international +41 22 372 9754. Fax: international +41 22 372 9777. E-mail: philippe.demoerloose@hcuge.ch

References


Disorders of Hemostasis

Plasma soluble fibrin monomer complex is a useful predictor of disseminated intravascular coagulation in neonatal sepsis

Disseminated intravascular coagulation (DIC) is a major factor influencing mortality in neonatal sepsis. Clinical trials have supported the use of antithrombin and activated protein C supplementation in DIC associated with sepsis.

Disseminated intravascular coagulation (DIC) is a major factor influencing mortality in neonatal sepsis. Clinical trials have supported the use of antithrombin and activated protein C supplementation in DIC associated with sepsis. The outcome of therapy was poorer with increasing DIC scores, suggesting that therapy could be more effective if initiated in the pre-DIC state than in established DIC. Wada et al. emphasized the usefulness of plasma soluble fibrin monomer complex (SMFC) in the diagnosis of DIC and pre-DIC state in adults. The present study was designed to assess the accuracy of plasma SMFC for early diagnosis of DIC in septic neonates.

Thirty-three neonates were prospectively enrolled in this study after obtaining informed consent from their parents. They were categorized into three groups: group I was formed of 10 healthy neonates as controls; group II comprised 13 neonates suffering from neonatal sepsis; and group III was formed of 10 septic neonates with overt DIC consecutively enrolled from those neonates of group II who satisfied the criteria proposed by the Scientific Subcommittee on DIC of the International Society on Thrombosis and Hemostasis for overt DIC during their clinical course. Plasma SMFC was measured according to the method previously reported by Wiman and Ranby using the Berichrome FM Kit from Dade Behring Marburg GmbH (Germany). The Kruskal Wallis H test was used to test for
The significance of differences between the plasma SFMC levels in the three studied groups. The Mann-Whitney U test was used to test for the significance of differences between the plasma SFMC levels in each of two groups. Receiver operating characteristics (ROC) analysis was used as a predictive value model to assess the diagnostic accuracy of the SFMC assay.

We demonstrated statistically significant differences of plasma SFMC levels between the three studied groups (p<0.001; Table 1). The level in septic neonates (33.69±11.85 mg/L) was significantly higher than in controls (24.5±6.09 mg/L) (p<0.05; Table 1). Furthermore, plasma SFMC level was significantly higher in septic neonates with overt DIC (73.2±31.55 mg/L) than in septic neonates (p<0.001; Table 1). These changes could be attributed to the activation of coagulation triggered by bacterial endotoxins causing enhanced thrombin generation and activity, since plasma SFMC is a molecular marker that is solely dependent on fibrin formation.

It has been demonstrated that the hemostatic system of neonates and preterm infants is generally shifted toward hypercoagulation, even in the absence of sepsis. During sepsis, however, this hypercoagulability is further exacerbated by decreases in factors inhibiting coagulation such as antithrombin, protein C and protein S, combined with the inhibition of fibrinolysis by PAI-1, which is reported to increase markedly in septic neonates. These unique hemostatic changes make septic neonates highly susceptible to developing DIC. Exhaustion of the natural blood coagulation inhibitory mechanisms with subsequent unopposed fibrinogen-converting activity of thrombin results in the establishment of overt DIC. Using ROC analysis we were able to define a cut-off value of 48.5 mg/L at which the sensitivity of plasma SFMC level for diagnosis of DIC in septic neonates was 100% with a specificity of 93% and overall accuracy of 97.5% (Figure 1). These findings document the high positive predictive value of plasma SFMC for the diagnosis of DIC. Accordingly, one can expect that septic neonates with a plasma SFMC level of 48.5 mg/L (critical level) or more are at risk of developing overt DIC if proper management is delayed. In support of this view, we interestingly demonstrated dramatic changes of SFMC plasma levels in a neonatal enrolled as a control and admitted to the neonates intensive care unit because of prematurity. The SFMC level was 22 mg/L at this stage. Unfortunately, the neonate then developed sepsis and finally DIC. The SFMC level rose to 42 mg/L at the stage of sepsis and to 70 mg/L with the development of overt DIC. These changes make SFMC an excellent marker for monitoring the magnitude of activation of coagulation in septic neonates and consequently could be used as an excellent prognostic marker. These findings are supported by those of Wada and co-workers who reported that plasma SFMC level correlates with DIC scores in adult patients. Furthermore, they demonstrated a significant reduction of plasma SFMC level after treatment in DIC patients who had a good outcome but not in those whose outcome was poor.

In conclusion, an elevated plasma level of SFMC has a high positive predictive value for early diagnosis of DIC in septic neonates. Serial monitoring of plasma SFMC levels with early identification of patients at risk of overt DIC might influence the morbidity and mortality of this devastating illness.

Tarek E. Selim,* Hayam R. Ghoneim,* Mohammad T. Khashaba, Sherin A. Rakha*

Departments of Clinical Pathology,* Pediatrics,* Al-Mansoura Faculty of Medicine, Al-Mansoura, Egypt

Key words: soluble fibrin, DIC, neonatal sepsis.

Correspondence: Tarek E. Selim, Hemostasis and Thrombosis Laboratory, Hematology Unit, Clinical Pathology Department, Al-Mansoura Faculty of Medicine, Al-Mansoura, Egypt. Phone: international +20.50.2247042. Fax: international +20.50.2267563. E-mail: Selim638@hotmail.com

References

Five prothrombotic polymorphisms and the prevalence of premature myocardial infarction

We studied 5 functional hemostatic polymorphisms in 281 patients with premature myocardial infarction and in 530 control subjects. The role of these polymorphisms when analyzed independently is small, if any. However, the simultaneous combination of factor XIII and prothrombin polymorphisms exacerbated the risk. (OR=12.12; p=0.028). Moreover, combinations of factor V Leiden with prothrombin, and factor XII with prothrombin polymorphisms were only identified in patients. Our results support the relevance of gene-gene interactions in myocardial infarction.

Table 1. Prevalence of classic cardiovascular risk factors and genetic polymorphisms in patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>MI patients</th>
<th>Controls</th>
<th>Adjusted p; OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>281</td>
<td>530</td>
<td></td>
</tr>
<tr>
<td>Age (Age of first MI)</td>
<td>44±6</td>
<td>50±19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>90</td>
<td>55</td>
<td>3.62(2.25-5.82)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>82</td>
<td>40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>29</td>
<td>27</td>
<td>2.45 (1.81-3.34)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11</td>
<td>12</td>
<td>0.420</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>70</td>
<td>22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FV Leiden Val34Leu</td>
<td>180</td>
<td>354</td>
<td>0.086</td>
</tr>
<tr>
<td>Val/Val</td>
<td>86±15</td>
<td>157±19</td>
<td>1.38(0.96-2.01)</td>
</tr>
<tr>
<td>Val/Leu/Leu</td>
<td>270</td>
<td>511</td>
<td>0.547</td>
</tr>
<tr>
<td>PT G20210A</td>
<td>267</td>
<td>520</td>
<td>0.241</td>
</tr>
<tr>
<td>FXII C46T</td>
<td>172</td>
<td>356</td>
<td>0.015</td>
</tr>
<tr>
<td>FXI Val33Ins</td>
<td>103±6</td>
<td>153±21</td>
<td>1.58(1.09-2.29)</td>
</tr>
<tr>
<td>FVII Del-323Ins</td>
<td>207</td>
<td>397</td>
<td>0.312</td>
</tr>
<tr>
<td>A1/A1</td>
<td>67±7</td>
<td>122±11</td>
<td>0.81(0.54-1.21)</td>
</tr>
</tbody>
</table>

Hypertension was defined as a systolic blood pressure > 140 mm Hg or a diastolic blood pressure > 90 mm. Hg on repeated observations over 3 months or, if no blood pressure values were available when the subject was under treatment with chronic antihypertensive therapy. Smoking habit was considered when the subject smokes more than 10 cigarettes per day. Hypercholesterolemia was defined as a total serum cholesterol level > 5.72 mmol/L (220 mg/dL). Diabetes was defined by a history of a fasting glucose of at least 7.8 mmol/L (140 mg/dL) or use of insulin or hypoglycemic medications.

Unprecedented efforts are underway to identify polymorphisms and to establish their relationship to susceptibility for common thrombotic disorders, especially myocardial infarction (MI). The influence of polymorphisms in MI is contradictory, with positive and negative results, although no study has identified a relevant risk (OR>4) associated with any polymorphism. Very recently we performed a study to test. Multiple analysis was performed using logistic regression adjusting for sex and cardiovascular risk factors (smoking habit, diabetes, hypertension, and hypercholesterolemia). Differences among groups for each individual test were considered significant when the uncorrected p<0.05. The strength of the association of major risk factors and polymorphisms with the occurrence of MI was estimated by calculation of the odds ratio (OR) and the Cornfield method for the calculation of 95% confidence intervals (CI). Gene interactions were determined by comparing the prevalence of combined carriership for 2 gene variants in patients and controls with respect to the whole population. There was no statistical deviation from Hardy-Weinberg equilibrium for any polymorphism in all groups of subjects. The frequencies of these polymorphisms in the control group did not differ from those reported in other Caucasian populations. Univariate analysis revealed that only the prothrombin polymorphism slightly increased the risk of premature MI (p=0.013; OR=2.73; 95% CI: 1.12-6.70).