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 at 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.[5] 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).[6] 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.[7-9] A discrepancy between antigen level and activity level was also observed for the French patient heterozygous for Thr575Met (FXI:Ag 42 U/dL).[6]

Myrna Germanos-Haddad, Philippe de Moerloose,° Françoise Boehlen,° Flora Peyvandi,# Marguerite 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, 1211 Geneva 14, Switzerland. Phone: international +41.22.3729751. Fax: international +41.22.3729777. E-mail: philippe.demoerloose@hcuge.ch

References

1. Salomon O, Seligsohn U. New observations on factor XI deficiency. Haemophilia 2004;10 Suppl 4:184-7.
2. de Moerloose P, Germanos-Haddad M, Boehlen F, Neerman-Arbez M. Severe factor XI deficiency in a Lebanese family: identification of a novel missense mutation(Trp501Cys) in the catalytic domain. Blood Coagul Fibrinol 2004;15:1-4
3. Neerman-Arbez M, Johnson K, Morris M, McVey JH, Peyvandi F, Nichols WC, et al. Molecular analysis of the ERGIC-53 gene in 35 families with combined factor V-VIII deficiency. Blood 1999;93:2253-60.
4. Alhaq A, Mitchell MJ, Sethi M, Rahman S, Flynn G, Boulton P, et al. Identification of a novel mutation in a non-Jewish factor XI-deficient kindred. Br J Haematol 1999;104:44-9.
5. Mitchell M, Cutler J, Thompson S, Moore G, Jenkins Ap Rees E, Smith M, et al. Heterozygous factor XI deficiency associated with three novel mutations. Br J Haematol 1999;107:763-5.
6. Quélin F, Trossaert M, Sigaud M, Mazancourt P, Mazancourt PD, Fressinaud E. Molecular basis of severe factor XI deficiency in seven families from the west of France. Seven novel mutations, including an ancient Q88X mutation. J Thromb Haemost 2004;2:71-6.
7. Saito H, Ratnoff OD, Bouma BN, Seligsohn U. Failure to detect variant (CRM+) plasma thromboplastin antecedent (factor XI) molecules in hereditary plasma thromboplastin antecedent deficiency: a study of 125 patients of several ethnic backgrounds. J Lab Clin Med 1985;106:718-22.
8. Mannhalter C, Hellstern P, Deutsch E. Identification of a defective factor XI cross-reacting material in a factor XI-deficient patient. Blood 1987;70:31-7.
9. Martincic D, Zimmerman SA, Russell E, Sun MF, Whitlock JA, Gailani D. Identification of mutations and polymorphisms in the factor XI genes of an African American family by dideoxy-fingerprinting. Blood 1998;92:3309-17.

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.

haematologica 2005; 90:419-421
(*http://www.haematologica.org/journal/2005/03/419.html*)

Disseminated intravascular coagulation (DIC) is a major factor influencing mortality in neonatal sepsis.[1] 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.[2] Wada *et al.* emphasized the usefulness of plasma soluble fibrin monomer complex (SFMC) in the diagnosis of DIC and pre-DIC state in adults.[3] The present study was designed to assess the accuracy of plasma SFMC 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.[4]

Plasma SFMC was measured according to the method previously reported by Wiman and Ranby[5] using the Berichrome FM Kit from Dade Behring Marburg GmbH (Germany). The Kruskal Wallis H test was used to test for

Table 1. Plasma levels of SFMC in the 3 groups studied.

Parameter	Group I (n=10) Mean±SD	Group II (n=13) Mean±SD	Group III (n=10) Mean±SD	P	P^1	P^2
SFMC (mg/L)	24.5±6.09	33.69±11.85	73.2±31.55	<0.001	<0.05	<0.001

P: significance of differences between the three studied groups; P^1: group II vs group I; P^2: group III vs group II.

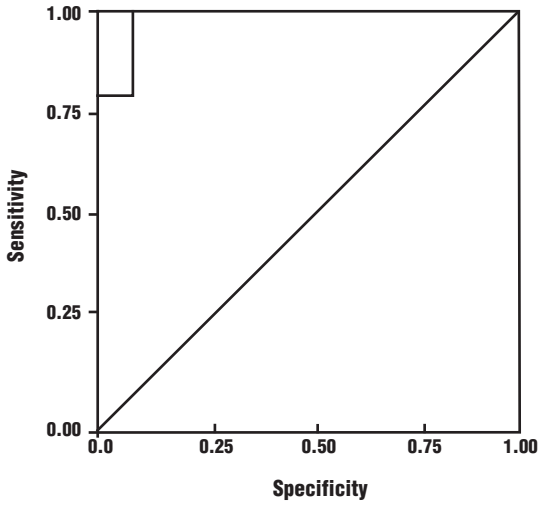

Figure 1. ROC analysis of SFMC for DIC. The large area under the curve represents the overall diagnostic accuracy of SFMC for DIC, estimated as 97.5%.

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.[6]

It has been demonstrated that the hemostatic system of neonates and preterm infants is generally shifted toward hypercoagulation, even in the absence of sepsis.[7] 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.[8] 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.[3]

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

1. Kreuz W, Veldmann A, Fischer D, Schlosser R, Volk W, Ettingshausen C. Neonatal sepsis. A challenge in haemostaseology. Semin Thromb Hemost 1999;25:531-5.
2. Levi M. Pathogenesis and treatment of disseminated intravascular coagulation in the septic patient. J Crit Care 2001;16:167-77.
3. Wada H, Wakita Y, Nakase T, Shimura M, Hiyoyama K, Nagaya S, et al. Increased plasma soluble fibrin monomer levels in patients with disseminated intravascular coagulation. Am J Hematol 1996;51:255-60.
4. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Hemost 2001;86:1327-30.
5. Wiman B, Ranby M. Determination of soluble fibrin in plasma by a rapid and quantitative spectrophotometric assay.Thromb Hemost 1986;55:189-93.
6. DeLa Cadena RA, Majluf-Cruz A, Stadnicki A, Agosti JM, Colman RW, Suffredini AF. Activation of the contact and fibrinolytic system after intravenous administration of endotoxin to normal human volunteers: correlation with cytokine profile. Immunopharmacology 1996;33:231-7.

7. Lao TT, Yin JA, Yuen PM. Coagulation and anticoagulation system in newborns. Gynecol Obstet Invest 1990;29:181-4.
8. Roman J, Velasco F, Fernadez F, Fernandez M, Villalba R, Rubio V, et al. Protein C, protein S and C4b binding protein in neonatal severe infection and septic shock. J Perin Med 1992;20:111-6.

Thrombosis

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.

haematologica 2005; 90:421-423
(*http://www.haematologica.org/journal/2005/03/421.html*)

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.[1] Very recently two big case/control studies suggested a minor role in MI for many polymorphisms.[2,3] The role of common genetic changes affecting candidate genes is certainly weak in itself, and much weaker than that of traditional cardiovascular risk factors. Like any complex and multigenic disease, MI probably involves many different factors that might interact to result in an additive or a synergistic co-effect. Therefore, an accurate evaluation of MI would require analysis of multiple polymorphisms and environmental risk factors in order to identify specific combinations with a significant association with the disease. Functional polymorphisms affecting hemostatic proteins could unbalance this system, increasing the risk of developing thrombotic or hemorrhagic diseases.

We studied five polymorphisms affecting the level or function of key hemostatic proteins in 281 patients who survived an acute MI before 45 years old during the last 7 years, and in 530 controls. The patients were selected from our Cardiology outpatient clinic and the controls were recruited from blood donors and traumatology and ophthalmology patients. A personal interview was done, and the clinical history of these controls was evaluated to discard vascular disease or personal history of thromboembolic or hemorrhagic disease. All the patients and controls were Caucasian.

The study had been approved by the local ethics committee, and was carried out in accordance with declaration of Helsinki. Univariate statistical analysis was performed by the χ^2 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),

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

	MI patients	*Controls*	*Adjusted p; OR (95%CI)*
N	281	530	
Age	44±6	50±19	
(Age of first MI)	(40±5)		
Male sex (%)	90	55	<0.001
			3.62(2.25-5.82)
Smoking (%)	82	40	<0.01
			2.45 (1.81-3.34)
Hypertension (%)	29	27	0.420
			0.85(0.57-1.26)
Diabetes (%)	11	12	0.172
			0.67(0.39-1.18)
Hypercholesterolemia (%)	70	22	<0.01
			6.62(4.59-9.54)
FXIII Val34Leu			
Val/Val	180	354	0.085
Val/Leu & Leu/Leu	86+15	157+19	1.38(0.96-2.0.1)
FV Leiden			
-/-	270	511	0.547
-/+ & +/+	10+1	19+0	1.31(0.54-3.16)
PT G20210A			
-/-	267	520	0.241
-/+	14	10	1.78(0.68-4.68)
FXII C46T			
C/C	172	356	0.015
C/T & T/T	103+6	153+21	1.58(1.09-2.29)
FVII Del-323Ins			
A1/A1	207	397	0.312
A1/A2 & A2/A2	67+7	122+11	0.81(0.54-1.21)

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.