albumin 2.5% final concentration. Before cryopreservation and after thawing, samples for cell counts, CD34 analysis, viability, clonogenic assays and microbiology were drawn directly from the bag. Cells were counted with a Sysmex K800 autoanalyzer (Toa Medical Electronics, Kobe, Japan). $CD34^+$ cells were quantified by flow cytometry (Procount, Becton Dickinson). Cell viability was assessed by ethidium bromide and acridine orange. Clonogenic assays were performed using a commercially prepared complete methylcellulose medium (Methocult GF H4434), supporting growth of CFU-GM, BFU-E and CFU-GEMM. Sterility controls were performed using an automated blood culture system (BacT/Alert: Organon Teknica) at 35ºC for 14 days. The Statistical Package for Social Science (SPSS) v. 8.0 was used to perform the statistical analysis. All studied variables showed a normal distribution. Means were compared using the t-test. A p value of <0.05 was considered statistically significant.

The mean collection volume without anticoagulant was 78 mL (range 40-137). At collection, mean values of total nucleated cells, $CD34^+$ cells and colony-forming units per bag were 4.28×10^8 (range 0.65-9.5), 1.94×10^6 (range 0.3-5.5) and 68.6×10^4 (range 14-197), respectively. Mean cell viability was 89.5% (range 56-95%). Results of thawing for non-programmed and programmed freezing are shown in the Table. There were no significant differences in mean recovery of $CD34^+$ cells, total nucleated cells or CFUs. Only one microbiological culture was positive for *S. viridans* in a thawed sample of a non-programmed cryopreserved unit.

These conclusions are similar to those obtained for peripheral progenitor cells[5,6] and suggest that non-programmed freezing of cord blood units is feasible, simplifying the method and decreasing costs in a cord blood bank. In spite of this, programmed freezing is probably recommended because it allows a temperature registry and is easier to standarize. Non-programmed freezing could be reserved for those banks that do not have adequate devices or in emergency situations. Besides, more studies are needed to determine whether there are clinical differences in grafting between cord blood units cryopreserved in these two different ways.

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Key words

Umbilical cord blood, cryopreservation, non-programmed freezing.

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Identification of one novel and three other point mutations in the protein C gene of five unrelated Brazilian patients with hereditary protein C deficiency

Hereditary protein C (PC) deficiency is an autosomally inherited disorder associated with a high risk of thrombosis.[1-5] We report three previously described point mutations[6] and one novel mutation (family E) in five Brazilian families with PC deficiency.

Sir,

According to the last PC gene database,[6] 160 mutations have so far been described. We determined the genetic basis of PC deficiency in five Brazilian patients with deep venous thrombosis, and their available relatives. The patients' mean age was 23 years (range 11 to 36).

Plasma PC levels (activity and antigen) were analyzed by coagulation (Bioclot PC Biopool) and *rocket* immunoelectrophoresis, respectively. Venous blood samples in EDTA were obtained for DNA analysis by polymerase chain reaction (PCR). The amplified fragments were submitted to SSCP and CSGE analysis for mutation screening. PCR fragments, which revealed an altered pattern were sequenced to determine molecular alterations. Results of the PC activity assays for the five families are shown in Table 1. In three patients the PC antigen test could not be performed. The other two patients had reduced levels of PC antigen, confirming type I deficiency.

Our results suggest a dominant autosomal pattern in relation to plasma PC deficiency and a recessive autosomal pattern regarding to clinical expression of thrombosis (Figure 1).

We identified four different substitutions (Table 1). The novel mutation is a 3190G to T transversion, converting Gly83 in Cys, in exon 5 affecting the first EGF domain. Most missense mutations in this domain are associated with a type I deficiency and

Table 1. PC activity assays (%) and identified mutations in subjects of the five Brazilian families with hereditary PC deficiency.

FAMILY A			FAMILY B			FAMILY C			FAMILY D			FAMILY E		
Subject	PC	Mutation 6182 C→T	Subject	PC	Mutation 6182 C→T	Subject	PC	Mutation 6246 G→A	Subject	PC	Mutation 1533A→G	Subject	PC	Mutation 3190G→T
I-f	50	+	I-f	26	+	I-m	56	+	II-s	29	+	I-f	107	-
I-m	126	-	I-m	120	-	II-s	106	-	II-b	95	-	I-m	54	+
II-s	139	-	II-*	34	+	II-*	38	+	II-*	48	+	II-b	70	+
II-b	101	-	II-b	33	+				II-b	28	+	II-s	82	-
II-*	47	+	III-ch	ND	+				II-b	70	+	II-s	73	-
												II-s	45	+
												II-*	63	+
												III-ch	48	+

Each member is identified by a generation number (Roman numeral). (m) mother; (f) father; (b) brother; (s) sister; (ch) child (son/daughter) in relation to the proband. The proband is identified by an asterisk (*). Normal range of PC activity in our laboratory is 72-142%. Low PC levels in patients and relatives cosegragate with the mutation and in all instances affected only one allele. (ND) no available data. Note: Nucleotide numbering as in Foster et al.[7]

could affect amino acid interactions leading to tertiary structure alterations and a consequent decrease in protein secretion. Considering that this mutation has not yet been described, we screened PCR products of one hundred neonates for this alteration which was absent in all of them. In addition, the alignment of part of the amino acid sequence of human PC and other species as well as of some vita-

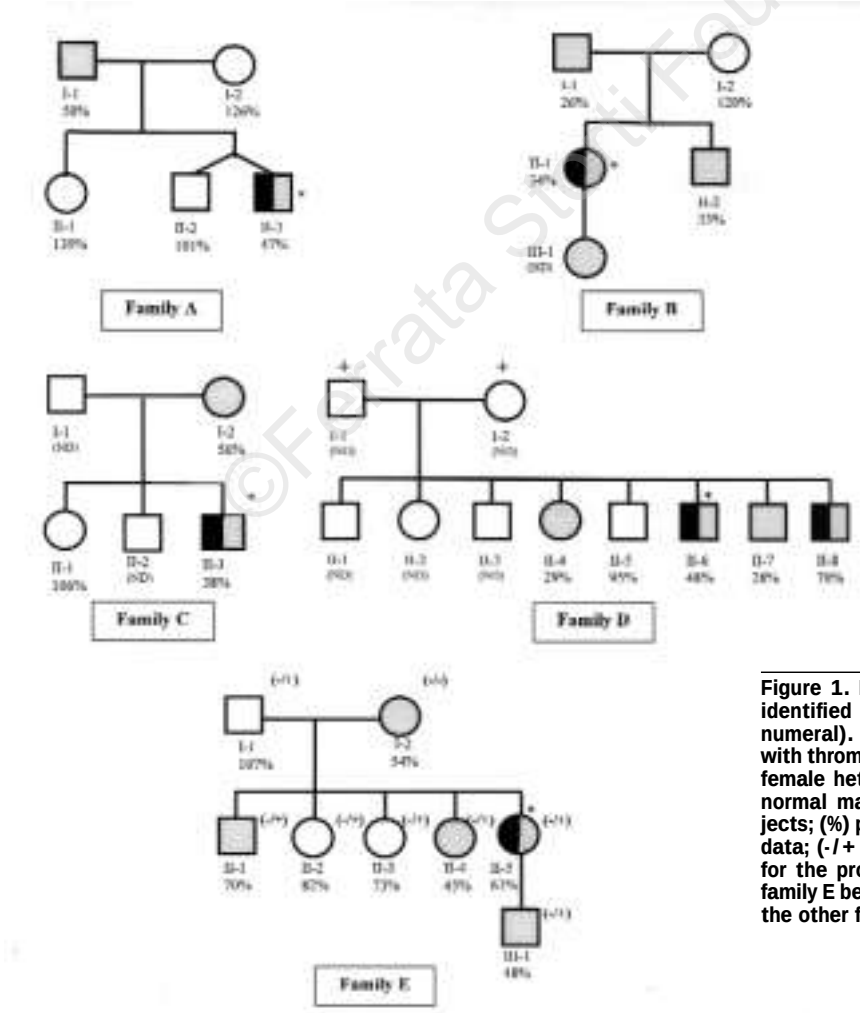

Figure 1. Family pedigrees. Each member is identified by a generation number (Roman numeral). *Proband; (■●) male and female with thrombotic complications; (◪◖) male and female heterozygous for PC deficiency; (□○) normal male and female; (+) deceased subjects; (%) plasma PC activity; (ND) no available data; (-/+ , -/-) heterozygous or wild genotype for the prothrombin variant is shown only in family E because this mutation was negative in the other families.

min-K dependent proteins, revealed that the affected amino acid was highly conserved, indicating an important structural or functional role.

The search for other established thrombophilic genetic risk factors such as protein S and antithrombin deficiency, factor V Leiden, methylene tetrahydrofolate reductase C677T variant, and prothrombin G20210A variant revealed that only one patient was heterozygous for the prothrombin variant (Figure 1). Family analysis revealed that individuals with the same mutation responsible for PC deficiency and prothrombin variant, had no clinical thrombosis. These data show that thrombosis can be the result of multiple acquired and genetic factors interacting with a consequent synergistic effect, possibly, including factors which still remain unknown.

In each family the deficiency cosegregates with the mutation (Table 1), indicating that the defect is the likely cause of PC deficiency in these patients.

Our results corroborate that recurrent mutations are very frequent in PC deficiency, since three of our identified mutations were previously described in other countries and two of them involved the hypermutable region of CPG dinucleotides.

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Oral anticoagulants and cyclosporin A

Cyclosporin A and oral anticoagulants have pharmacologic interactions. In this letter we report a case of angioimmunoblastic T-cell lymphoma with a concomitant deep venous thrombosis in which a negative interaction between warfarin and cyclosporin A was observed. The introduction of cyclosporin A required a larger dose of warfarin in order to reach the therapeutic level.

Sir,

A number of drugs can influence both cyclosporin A (CsA) blood levels and course of the oral anticoagulant treatment (OAT) with a synergistic or antagonistic effect.[1]

Few observations exist about the interaction between CsA and oral anticoagulants (OAs). We report here a case of a 65-year old woman who was given CsA while receiving warfarin for venous thromboembolism. In August 1996 a diagnosis of angioimmunoblastic T-cell lymphoma was made and six courses of standard CHOP regimen were given. In November 1996 while still on chemotherapy, the patient developed a deep vein thrombosis (DVT) of the left leg. Low molecular weight heparin was given for two months and DVT regressed. In February 1997 a CT scan of the abdomen showed an asymptomatic vena cava thrombosis spread from the iliac bifurcation to the renal veins and long-term warfarin therapy was started. In October 1997, because of a lymphoma relapse, CsA was introduced at the dose of 300 mg p.o. bid, in order to control the malignant T-cell proliferation as suggested by Advani *et al*.[2] After the beginning of CsA therapy, INR decreased about 40% in several repeated controls, and a larger dose of warfarin had to be administered (from 18.75 mg to 27.50 mg/week, progressively). Thereafter, for a given dose of warfarin, when CsA blood levels remained within the therapeutic range the INR values became stable with the same OA dose. When OAT was withdrawn, CsA blood levels remained unchanged.

To our knowledge, there are only two reports about interactions between CsA and OAs. In the first[3] the patient was receiving warfarin and phenoarbital as well, a drug known to enhance the activity of cytochrome P450[4] and consequently to reduce the pharmacologic effect of both CsA and warfarin. Because of this negative interaction, the dosage of both CsA and warfarin had to be increased. This is at variance with our case in which only the dose of warfarin had to be modified, and the difference can be ascribed to the concomitant use of phenobarbital. In the second report[5] the patient was on acenocoumarol and CsA displayed a potentiating effect of this OA, thus making it necessary to reduce the dosages of both CsA and OA. The reason for this opposite behavior (positive interaction) cannot be explained by the different half-lives: 10 hours for acenocoumarol and 36 hours for warfarin. A different metabolism of acenocoumarol could be assumed with regard to the hydroxylating action of cytochrome P450.[4]

A clinical setting in which there is indication for CsA