

74.8 U/dL; range 71-103 U/dL) levels (Table 1).

In patient #1 we utilized desmopressin (0.3 µg/kg s.c. starting 60 min before the operation) 12 hourly on the day of the operation, then once daily until day +4. We gave DDAVP once daily (0.3 µg/kg s.c.) for 5 days to the remaining patients, starting on the day of the operation. All patients received tranexamic acid (10 mg/kg thrice daily i.v. for 5 days). Serum electrolytes were monitored regularly.

DDAVP therapy was well tolerated and no hemorrhagic complications occurred during or after surgery.

FXI deficiency is a rare inherited coagulation disorder characterized by rarity of spontaneous bleeding but the risk of severe hemorrhagic complications after trauma or surgery. There is often little direct correlation between the tendency to bleed and the severity of the disease itself, so it is extremely difficult to predict hemorrhagic complications after surgery in patients with mild disease.<sup>1-3</sup>

Currently available therapeutic products for the treatment of bleeding in FXI deficient patients include fresh frozen plasma and virus-inactivated FXI concentrates: the former may carry blood-borne viruses, the latter, although the first choice treatment in patients with severe FXI deficiency, should be used cautiously because of its thrombotic risk.<sup>4-6</sup>

Recent reports indicate that DDAVP has been used successfully to prevent surgical bleeding in FXI defective patients.<sup>7,8</sup> Our case reports confirm these findings: we first tested and then utilized subcutaneous desmopressin in symptomatic heterozygous FXI deficient patients undergoing surgery. No hemorrhagic complications occurred peri-operatively.

It remains to be clarified how DDAVP acts in such patients: the administration of DDAVP causes a slight increase in FXI activity and a marked increase in FVII/VWF levels with normalization of APTT. Even though the observed APTT normalization is probably linked to the marked increase in FVIII:C, the slight but significant increase in FXI activity may contribute to the hemostatic efficacy of DDAVP in such patients.

Spurious increases of FXI:C in functional assays due to concomitant, DDAVP-dependent increases of FVII:C are rather unlikely as Castaman *et al.*<sup>7</sup> have previously demonstrated parallel degrees of increase in both FXI:C and FXI:Ag after administration of DDAVP.

However, although the mechanism by which DDAVP increases FXI levels is still not clear, our data suggest that this drug is effective in preventing surgical bleeding in patients with mild factor XI deficiency.

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### Comparison between radial immunodiffusion and flow cytometry techniques for detecting antiplatelet antibodies

The aim of our work was to compare radial immunodiffusion (RI) (in use for years) versus flow cytometry (FC) (a new technique). The discrepancies of the results in our patient population indicate that both techniques are valuable tools to understand the pathogenesis of thrombocytopenia.

Sir,

To evaluate the clinical use of RI and FC for detecting antiplatelet antibodies, we analyzed platelet samples from 39 patients. The samples were grouped according to the etiology of the thrombocytopenia into: group A (n=12): immune thrombocytopenic purpura (ITP) (Table 1), group B (n=19): conditions associated to bone marrow failure or malignancy (Table 2) and group C (n=8): unexplained mild thrombocytopenia.

Surface platelet associated IgG (PAIgG) was assayed by FC using the procedure described by Lin *et al.*<sup>1</sup> and total (PAIgG) by RI using the procedure described by Morse *et al.*<sup>2</sup> We observed significant positive correlations between both methods when all 39 cases were examined (r=0.44, p=0.006). The main contribution to this significant positive correlation was given by group A results (r=0.8, p=0.0018).

**Table 1. Results of platelet antibody tests in Group A.**

PT.	RI	FC	Clinical diagnoses (comments)
1	4	44	ITP (C-I remission, normal platelet count)
2	5	18	ITP (C-I remission, normal platelet count)
3	2.5	19	ITP (C-I remission, normal platelet count)
4	3	34	ITP (past history of ITP/pregnancy)
5	6	24	ITP (past history of ITP)
6	5	26	ITP (past history of ITP/pregnancy)
7	9	47	ITP (taking corticosteroids, low platelet count)
8	9	67	ITP (taking corticosteroids, low platelet count)
9	15	32	ITP (taking corticosteroids, low platelet count)
10	9	49	ITP (antiphospholipid syndrome, low platelet count)
11	8	50	ITP (taking corticosteroids, low platelet count)
12	38	96	ITP (taking corticosteroids, low platelet count, autoimmune thyroiditis)

FC is measured as median of arbitrary fluorescence intensity units (FIU). C-I: corticosteroid-induced. The normal surface PAIgG level was  $33 \pm 22$  FIU. This value is the mean of the median values  $\pm 2SD$  for FC (n=94). RI is expressed in femtograms per platelet (fg/plt). The normal total IgG value was below 7 fg/plt using RI (n=20).

**Table 2. Results of platelet antibody tests in group B.**

Pt.	RI	FC	Clinical diagnoses (comments)
1	6.5	35	Myelodysplasia
2	1.5	20	Myelodysplasia
3	6	20	Myeloproliferative syndrome
4	4.5	30	Lymphoproliferative syndrome
5	11	196	Lymphoproliferative syndrome
6	6	25	Lymphoproliferative syndrome
7	4	43	Paroxysmal nocturnal hemoglobinuria
8	24	57	Paroxysmal nocturnal hemoglobinuria
9	1.5	32	Aplastic anemia
10	6	26	Hypersplenism
11	9	25	HIV
12	15	44	HIV/HCV
13	6	22	HCV
14	6	25	HCV
15	11	35	HCV
16	14	22	Hepatitis
17	38	74	Hepatitis
18	8	28	Hepatitis
19	5	54	Gestational thrombocytopenia

FC is measured as median of arbitrary fluorescence intensity units (FIU). The normal surface PAIgG level was  $33 \pm 22$  FIU. This value is the mean of the median values  $\pm 2SD$  for FC (n=94). RI is expressed in femtograms per platelet (fg/plt). The normal total IgG value was below 7 fg/plt using RI (n=20).

In group A, although no patient had a recent diagnosis of ITP, the low platelet counts suggested an active disease. RI gave more abnormal results (6 of 12 patients) than FC (2 of 12 patients); the discrepancy between the methods is attributed to a high platelet turnover, yielding younger platelets with more IgG due to higher content of  $\alpha$ -granules. Among these six patients we also found two patients with abnormal FC. This indicates the specificity of the FC method. Corticosteroid-induced remission or

past history of ITP explains the normal results obtained by both methods in this group.

Three patients in group B gave abnormal results by both RI and FC. One patient had chronic lymphoid leukemia and a clinical picture of ITP. Another had paroxysmal nocturnal hemoglobinuria with severe thrombocytopenia; platelet kinetic studies showed decreased platelet production and, in spite of the normal platelet survival during treatment with corticosteroids and cyclosporin, we cannot exclude ITP. The patient probably had a combined mechanism explaining her thrombocytopenia. The third patient suffered from hepatitis of unknown serology.

The abnormal RI results with normal FC found in group B could be explained by an increased plasma IgG concentration causing elevated platelet  $\alpha$ -granule IgG in liver disease.<sup>3</sup> The clinical and laboratory findings of our HIV patients were not consistent with ITP implying that RI abnormalities are unspecific findings. The FC method proved specific for detecting platelet immune destruction.

All group C patients gave normal results using both methods (data not shown).

RI proved to be as good a marker for the intensity of thrombopoiesis as the reticulated platelet count. This last method also detects the dense granular pool of nucleotides, which appeared to cause a substantial proportion of non-specific labeling.<sup>4</sup> Noris *et al.* concluded that there was no direct relation between platelet age and thiazole orange fluorescence (TO) of platelets and, that the greater TO is largely dependent on the increased platelet volume.<sup>5</sup> RI is also useful as a marker for thrombopoiesis, and not only for immune platelet destruction as is the case with FC. Moreover, RI can discriminate between the active and remission phases of ITP.

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### Key words

Radial immunodiffusion, flow cytometry, PAIgG.

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### G20210A homozygosity in antiphospholipid syndrome secondary to systemic lupus erythematosus

We report the first case of systemic lupus erythematosus (SLE)-associated antiphospholipid syndrome in a young female homozygous for the G20210A allele in the prothrombin gene who developed an extensive venous thrombosis while taking oral contraceptives.

Sir,

The risk of deep venous thrombosis (DVT) is increased by conditions that cause hypercoagulability or venous stasis.<sup>1</sup> A variant of prothrombin (G20210A) represents the second most common genetic risk factor in Caucasians, after factor V Leiden.<sup>2-7</sup> The mechanism of thrombosis is probably related to the high amounts of thrombin generated.<sup>2</sup>

We report a case of a 28-year old woman who developed an extensive DVT after having taken oral contraceptives for one year. Venous ultrasonography demonstrated a femoral-iliac thrombosis with proximal extension to the common iliac vein. Past history was positive for oral ulcers and Raynaud's phenomenon, since she was a teenager. She reported photosensitivity lasting years, with an important episode on the scalp some months earlier: scarring lesions with atrophy and alopecia were still evident. Platelet count, partial thromboplastin and prothrombin time, antithrombin III and fibrinogen were normal. The patient was treated with continuous intravenous non-fractionated heparin infusions followed by oral warfarin for 7 months (INR = 3.0). During the follow up erythrocyte sedimentation rate and immunoglobulin levels were moderately increased and white cell count repeatedly low. Her immunologic profile showed positive antinuclear-antibodies, anti-DNA, SSA, anticardiolipin, anti- $\beta_2$ GPI and C4 hypocomplementemia. A second set of tests a few months later confirmed the picture. The final diagnosis was DVT in a patient with APS and SLE.

The search for factor V Leiden was negative. Prothrombin G20210A was evaluated by PCR and Hind III digestion. The patient was a G20210A homozygote. Family members were asymptomatic and none had a history of thrombosis. Their genotypes are given in Figure 1.

The G20210A mutation is associated with DVT

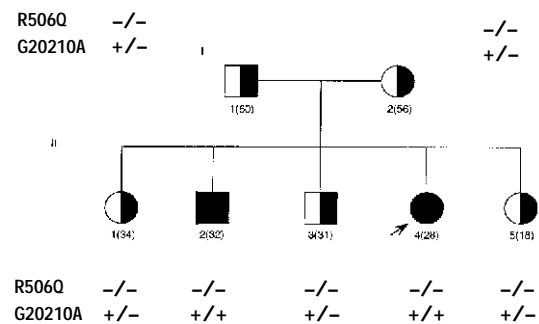


Figure 1. Pedigree of the family studied. The age of each subject is shown in brackets. The results of the mutations studied and their inheritance is indicated.

with a 2.8 fold independent risk.<sup>2</sup> Homozygotes are rare; although they present the highest prothrombin activity values<sup>8</sup> data on their risk of thrombosis are controversial. This is not unexpected considering that a thrombotic event is the manifestation of a multifactorial disease.<sup>1</sup> The patient described here had two acquired factors associated with a genotype at risk: APS and oral contraceptives. The relative risk in women with factor V Leiden using contraceptives is 34.7; that of carriers of prothrombin variant is unknown.<sup>1</sup>

APS is characterized by venous and arterial thrombosis and often by recurrent fetal loss in the presence of lupus anticoagulant, anticardiolipin antibodies or both. It has been proposed that anti- $\beta_2$ GPI, the presence of which correlates strongly with thrombosis, should be included in the APS biological score.<sup>9</sup> In APS associated with SLE the risk of DVT is enhanced by the possible vasculitis process inherent to disease activity. The frequency of factor II mutation is not expected to be increased in patients with APS but, in analogy to that which occurs for factor V Leiden,<sup>10</sup> when present may represent an independent risk factor for thrombosis.

The other members of the family had no history of DVT. The G20210A heterozygous parents are free of events, despite I-2 having had 5 pregnancies, a condition known to favor thrombosis. Neither has II-2, a G20210A homozygote, had any events, but this subject has never been exposed to risk situations.

In conclusion, our study suggests that the thrombotic risk in G20210A variant is mild and requires additional factors to become manifest.

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#### Key words

Thrombophilia, prothrombin variant, antiphospholipid syndrome, SLE