Table 1. Initial patient characteristics and results of treatment with anti-D (no.= 10). ***SD, standard deviation.***

	Mean (± SD)	*Median*	*Range*
Patient age (years)	5.0±3.2	4.6	0.8-11.5
Initial platelet count (per µL)	4,000±2,900	2,500	1,000-8,000
Anti-D dose (µg/kg)	49.6 ± 5.8	50.8	35.1-56.4
Time to platelets ≥ 20,000/µL (hrs)	22.3 ± 11.4	16.4	12-39.5
Peak platelet count (per µL)	262,000±202,000	253,000	20,000-689,000
Drop in hemoglobin (g/dL)	1.27±0.7	1.2	0.4-2.5
Retreatment	3 patients at 10, 16 and 65 days		

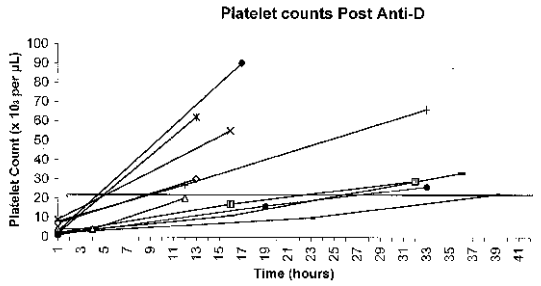

Figure 1. Platelet counts over the first 40 hours after anti-D (no.=10).

or prednisone (4 mg/kg/day orally, tapering off by day 21). Both the low and high dose IVIG arms were superior to anti-D in mean time to platelet count > 20,000/µL: 1.4 versus 2.9 versus 3.9 days, respectively. Tarantino *et al.*[10] retrospectively compared children receiving 0.8-1 g/kg IVIG (N=14) or 45-50 µg/kg anti-D (N=13) and reported a mean time to platelet count ≥ 20,000/µL of 1.26±0.82 days and 1.54±0.51 days. Although the number of patients in both our study and the study by Tarantino is small, the use of a single dose of 50 µg/kg rather than two daily doses of 25 µg/kg may have been the cause of the improved response time.

In conclusion, a single 50 µg/kg intravenous dose of anti-D produced a rapid increase in platelet count in children with newly diagnosed acute ITP. A randomized trial comparing higher doses of anti-D to IVIG in children with acute ITP appears warranted.

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Key words

Immune thrombocytopenic purpura, anti-D, children

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Treatment of refractory ITP with extracorporeal immunoadsorption over a protein-A sepharose column: a report of two cases

Two females with refractory ITP underwent plasma immunoadsorption over protein A-sepharose columns. The immediate response to immunoadsorption was unsuccessful while anti-platelet and anti-HLA antibodies disappeared from serum. However platelets progressively rose to normal in the following months, medical therapy was gradually withdrawn and the patients remain in remission so far.

Sir,

Extracorporeal immunoadsorption of antibodies over a protein A-silica matrix (Prosorba®, USA) has been recently proposed among second line therapy for refractory chronic immune thrombocytopenia (ITP).[1-3] Plasma immunoadsorption over protein A-sepharose columns (Excorim/Citem 10 (EC10®),

Excorim, Lund, Sweden) is a two column system that allows the processing of larger amounts of plasma during each procedure as compared to Prosorba.[4] While EC10 proved to be effective in the removal of acquired inhibitors to factor VIII or factor IX,[4] there are no reports about refractory ITP.

Patient #1. A 67-year old female affected by chronic ITP was unsuccessfully given steroids, high dose immunoglobulins (IVIgG), danazol and two courses of vincristine. Only a transitory response occurred after splenectomy and symptomatic thrombocytopenia persisted despite treatment with plasmapheresis, azathioprine 100 mg/day, and then mesterolon and cyclophosphamide 50 mg/day each. She was, therefore, offered experimental treatment with EC10. Her platelet count was 24×10^9/L, platelet-associated immunoglobulins (PAIgG) as demonstrated by direct immunofluorescence were increased and anti-platelet GPIIb-IIIa autoantibodies (GTI Pak-Plus, WI, USA) were detectable in the serum.

She underwent 3 immunoadsorption procedures over one week and about five liters of plasma were processed, while she was still on prednisone and mesterolon 50 mg/day (Figure 1a). IgG level decreased from 9.72 g/L to 0.52 g/L at the end of the third procedure, when she received 90 g of IVIgG. Her platelet count rose to 80×10^9/L, but returned to the basal value 13 days later, while PAIgG were still detectable; by contrast, anti-platelet GPIIb-IIIa autoantibodies disappeared from the serum. Platelet count then progressively rose to normal in the following 6 months; prednisone and mesterolon were tapered until withdrawal and the patient remains in complete remission so far.

Patient #2. A 71-year old female suffered from severe symptomatic thrombocytopenia (platelets 3×10^9/L) despite treatment with steroids, IVIgG, and vincristine; PAIgG were increased without serum specific anti-platelet autoantibodies while anti-HLA class I antibodies were detected in the serum. Splenectomy was ruled out because of concurrent personal risk factors. Therapy with danazol was started and the patient underwent EC10 treatment as previously described. About 10 liters of plasma were processed during the immunoadsorption while IgG decreased from 13.1 g/L to 1.29 g/L and anti-HLA antibodies disappeared from the serum. The patient became responsive to platelet concentrates transfused during the first two procedures, and exhibited a transient increase in platelet count (23×10^9/L) when she was

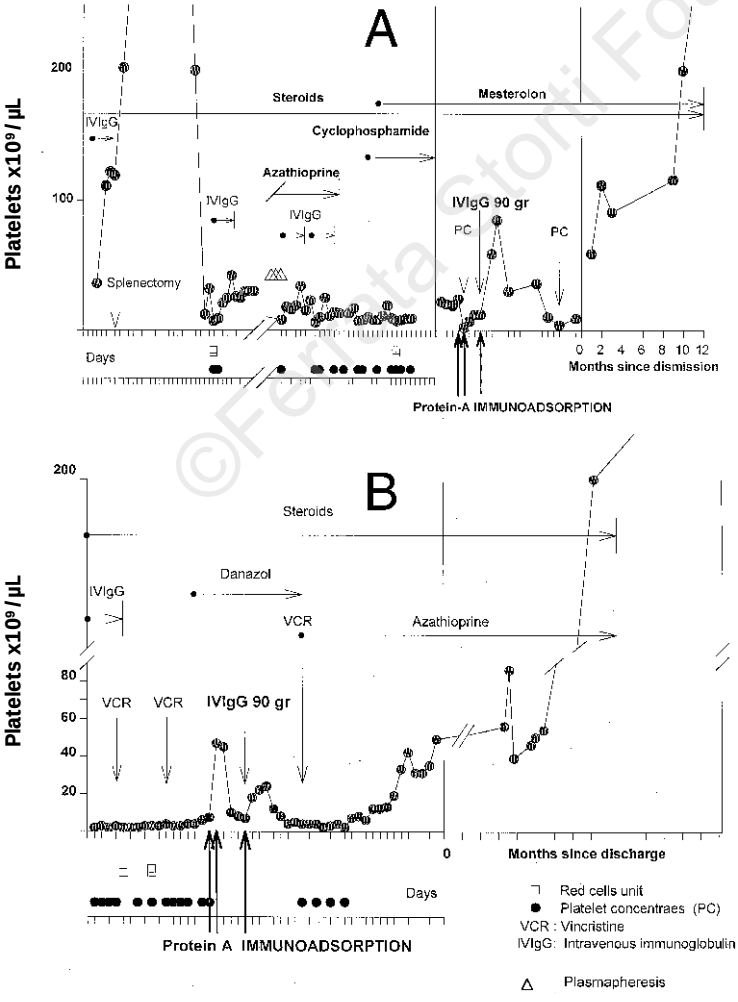

Figure 1. Clinical course of the patient.

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given 90 grams of IVIgG. Eight days after immunoadsorption, danazol was replaced by azathioprine and a third course of vincristine was given. Six days later, the platelet count began to rise. Pharmacologic therapy was tapered down until withdrawal and the patient is in complete remission so far.

Snyder *et al.* reported in 1992 a durable response in 36% of refractory ITP patients treated with Prosorba columns. The clinical response was associated with a significant decrease in specific serum platelet autoantibodies, PAIgG and circulating immune-complexes (CIC).[2] In these only two cases we have treated, we observed the immediate disappearance of anti-platelet GPIIb-IIIa and anti HLA class I antibodies from serum while the clinical response was time-delayed. However, the significance of removing circulating platelet autoantibodies in chronic ITP is questionable, since fewer than 50% of ITP patients have detectable antibodies in the serum and many of them are non-pathogenic. The immunomodulatory effect of the immunoadsorption is a better explanation of the late response observed in our patients. However, further studies are required to explain and validate the use of immunoadsorption treatment in refractory ITP.

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Protein-A column, immune thrombocytopenia, platelet autoantibodies.

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Programmed versus non-programmed freezing of umbilical cord blood

Programmed freezing is an expensive procedure that requires the use of sophisticated equipment, not available in many centers. We designed a prospective study to compare programmed and non-programmed freezing for cord blood. Our results suggest the feasibility of non-programmed freezing for umbilical cord blood, simplifying the method and decreasing costs in a cord blood bank.

Sir,

Many authors have established the optimal conditions for cryopreservation of umbilical cord blood to be a controlled cooling rate of 1°C/min.[1-3] However, programmed freezing is an expensive procedure that requires the use of sophisticated equipment, not available in many centers.

We designed a prospective study to compare programmed and non-programmed freezing for cord blood. For this purpose, 39 cord blood units were collected, volume reduced and cryopreserved in two 25 mL aliquots with 10% DMSO final concentration, following Rubinstein's method.[4] One of the aliquots was cryopreserved in a controlled rate freezer (Planer Biomed, Kryo 10) with a cooling-rate of 1ºC/min, and the other one was placed directly into a –80ºC mechanical freezer (Koxka). After 24 hours, the –80ºC frozen cord blood was stored in a liquid nitrogen tank in the vapor phase. After 7 days, the UCB was thawed by submerging the bag in a 37ºC water bath and washing the cells with thawing solution containing dextran and human

Table 1. Recovery of nucleated total cells, CD34⁺ cells and colony-forming units after thawing.

	N	*Mean*	*Median*	*SD*	*Min*	*Max*	*p*
TNC x10^8							
-80°C	40	3.76	3.6	1.63	0.67	7.8	
-120°C	38	3.57	3.32	1.6	0.69	8.3	0.541
CD34 x10^6							
-80°C	40	1.79	1.4	1.48	0.18	8.1	
-120°C	38	1.55	1.3	1.02	0.17	4.7	0.498
CFUs x10^4							
-80°C	39	43.85	34.91	34.15	2.1	127	
-120°C	33	40.21	27.05	34.55	2.1	131.3	0.278
TNC Rec (%)							
-80°C	40	88	89	34.15	61.1	150	
-120°C	38	85.6	84.24	11.22	59.09	110	0.109
CD34 Rec (%)							
-80°C	33	98.8	85	41.83	25	175	
-120°C	31	92.57	90	33.05	32.14	166.67	0.421
CFUs Rec (%)							
-80°C	28	70	69.69	39.55	11.49	151.43	
-120°C	22	53.36	45.55	36.94	5.96	140	0.199
Viability (%)							
-80°C	32	71	74	11.03	46	87	
-120°C	30	69.13	69.5	13.72	38	94	0.461

CNT: total nucleated cells. CFUs: colony-forming units. Rec: recovery expressed as percentage.

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