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# Protein A Sepharose immunoadsorption can restore the efficacy of platelet concentrates in patients with Glanzmann's thrombasthenia and anti-glycoprotein IIb–IIIa antibodies

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**Summary.** Type I Glanzmann's thrombasthenia is a rare congenital platelet function disorder, characterized by undetectable platelet membrane glycoprotein IIb–IIIa (GPIIb–IIIa). Severe bleeding is controlled by transfusion of normal platelets, leading in some cases to the occurrence of anti-GPIIb–IIIa isoantibodies, which induces a loss of transfused platelet efficacy. We used immunoadsorption on protein A Sepharose (IA-PA), which has been shown to be efficient in decreasing the titre of antibodies in several immune diseases, in three patients with Glanzmann's thrombasthenia and anti-GPIIb–IIIa isoantibodies on five different occasions. IA-PA was well tolerated with no deleterious side-effects reported. It induced a dramatic decrease of total immunoglobulin (Ig)G, including anti-GPIIb–IIIa isoantibody levels, as assessed by the monoclonal antibody-specific immobilization of platelet antigens test and the *ex vivo* inhibition of normal platelet aggregation induced by the patient's platelet-rich or platelet-poor plasma. Elimination of the antibody was associated with a correction of the bleeding time following platelet transfusion. IA-PA combined with platelet transfusion made it possible to control two life-threatening haemorrhages, and allowed two surgical procedures and one bone marrow transplantation to be performed safely. Our experience suggests that IA-PA, which restores the haemostatic efficacy of platelet transfusion, is a valuable therapeutic strategy in patients with Glanzmann's thrombasthenia and anti-GPIIb–IIIa isoantibodies.

**Keywords:** Glanzmann's thrombasthenia, anti-glycoprotein IIb–IIIa isoantibodies, platelet transfusions, immunoadsorption on protein A-Sepharose, platelet glycoprotein GPIIb–IIIa.

Type I Glanzmann's thrombasthenia (GT) is a severe constitutional, recessively inherited bleeding diathesis, resulting from the absence of the platelet fibrinogen receptor, the glycoprotein (GP) IIb–IIIa complex. It is characterized by an undetectable (< 5%) level of GPIIb–IIIa or of platelet fibrinogen (Caen *et al*, 1966; Nurden & George, 2001).

Severe bleeding episodes, including intracerebral haemorrhages, epistaxis, voluminous haematomas, usually occur with an onset in early childhood (George *et al*, 1990). Menorrhagias are of particular severity, often requiring continuous hormonal therapy. Platelet transfusions correct the bleeding time and can control and/or prevent bleeding. However, anti-GPIIb–IIIa isoantibodies may occur, inhibiting the donor's platelet plug formation at the bleeding site, which can transform bleeding episodes or surgery into life-threatening events (Bellucci *et al*, 2000). Among the different therapeutic strategies available, we used immunoadsorption on protein A sepharose (IA-PA), which has been shown to be an efficient extracorporeal technique for specific removal of immunoglobulin (Ig)G or circulating immune complexes (Gjörstrup & Watt, 1990). As such, it can decrease the level of antibodies in different models of autoimmune-related diseases (Snyder *et al*, 1992; Gutensohn *et al*, 1998; Benny *et al*, 1999; Braun *et al*, 2000; Guillet *et al*, 2001) as well as in patients with immune-mediated renal disorders, or

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anti-human leucocyte antigen (HLA) antibodies before renal transplant (Kriaa *et al*, 1995). Furthermore, the haemostatic efficacy of replacement therapy has been restored in some patients with haemorrhagic disorders and alloantibody-related refractoriness to substitutive therapy (Kiss *et al*, 1989; Boughton *et al*, 1990; Gailani, 1992; Watt *et al*, 1992). We have successfully treated, on five different occasions, three patients with type I GT and anti-GPIIb–IIIa isoantibodies. We have shown that in case of life-threatening bleeding or before invasive procedures, IA-PA can restore the haemostatic efficiency of platelet concentrates. The clinical benefit and shortening of the bleeding time observed after platelet transfusion correlated with a decrease in serum total IgG and more specifically in anti-GPIIb–IIIa antibody. Control of severe bleeding and safe major surgery could be achieved using platelet transfusions in all patients.

## MATERIALS AND METHODS

Platelet count was performed with an H2 Analyser (Bayer Diagnostics, Puteaux, France). Bleeding time was performed according to the Ivy quantitative technique. Normal range was 2–4 min, with blood volume < 120 μl.

*Platelet aggregation study.* Blood was collected on 3·8% sodium-citrate (1/9 volume). Platelet-rich plasma (PRP) was obtained after centrifugation at 200 ***g*** for 15 min at 15°C, and platelet-poor plasma (PPP) by a second centrifugation at 3000 ***g*** for 15 min at 15°C. The tests were performed at 37°C as previously described (Han & Ardlie, 1974), using an aggregometer (Chronolog Coultronics®, Margency, France) and $5{\cdot}10^{-5}$ mol/l adrenaline from Sigma, St Quentin-Fallavier, France, 2·5 mg/l collagen from Hormon Chemie, Munich, Germany.

The antibody inhibiting activity was assessed, measuring the *ex vivo* inhibition of ADP and collagen-induced aggregation in 1/1 mixtures of control PRP with different dilutions of patient's PPP or in a 4/1 mixture of control/patient's PRP. The final concentration of platelets was $250 \times 10^9$/l in all the mixtures. The decrease in aggregation velocity was expressed as a percentage of aggregation of the control PRP, and the inhibitory activity calculated using the following formula:

$$\text{Inhibitory activity (\%)}: = \frac{(\text{control} - \text{mixture})\ \text{aggregation velocity} \times 100}{\text{control aggregation velocity}}$$

The inhibitory activity was considered to be relevant when ≥ 25%.

*The extracorporeal procedure for protein A sepharose immunoadsorption.* The procedure was performed as previously reported (Gjörstrup & Watt, 1990; Guillet *et al*, 2001), using a plasma flow monitor (Citem 10®; Excorim) and two parallel 62·5 ml sterile columns (Immunosorba Protein A®; Excorim, Lund, Sweden). Briefly, the patient's citrated blood is passed through a plasma separation device. Cell-free plasma is then directed to one of two Sepharose/protein A columns, which electively binds the IgG. While one column binds IgG from plasma, the other is regenerated by serial elutions and washes. Upon exit from the column, the IgG-free plasma is mixed with the blood cells to be returned to the patient.

Generally, three plasma volumes were treated during each IA-PA procedure. The procedure frequency was usually three per week, but could be increased to once a day in case of emergency.

Replacement therapy with polyvalent immunoglobulins was used after the last IA-PA in most patients, to avoid the possible side-effects of decreased IgG levels; 0·4 g/kg IgG (Tegelin®; LFB, Les Ulis, France) were infused intravenously in four out of five patients. In one patient, this infusion was performed after the penultimate procedure.

*Platelet concentrates (PC).* Leucodepleted-pooled platelet units (PU) originating from several donors were infused to patients 1 (case 1B) and 3 (case 3A). Leucodepleted PC obtained from single donor aphereses were infused in the remaining patients. All PC units were irradiated prior to infusion for the patient undergoing bone marrow transplantation. One platelet unit was defined as $0{\cdot}5 \times 10^{11}$ platelets.

*Assessment of IA-PA biological efficacy.* Quantitative bleeding time was performed 30 min after PC infusion. Ig serum levels were determined before and after each procedure using a Beckman nephelometric analyser and anti-human IgG, IgA, IgM, from Beckman (Furleton, USA). The normal ranges were: IgG 7–17 g/l, IgA 0·7–3·8 g/l, IgM 0·7–2·1 g/l.

Serum anti-GPIIb–IIIa isoantibody was assessed according to the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) technique (Kiefel *et al*, 1987; Tchernia *et al*, 1993). Sera were considered as positive if the optical density (OD) was > 0·2. In one patient, the antibody level was assessed by serial dilutions with the same technique.

### *Patients*

The three patients were two males, aged 42 and 6 years (patients 1 and 3), and a girl aged 14 years (patient 2). All three had a history of bleeding and had been diagnosed with type I GT. Acquired clinical refractoriness to platelet concentrate infusions led to evidence of anti-GPIIb–IIIa isoantibodies.

*Case 1A.* Type I GT had been diagnosed at age 19 years and dental surgery required PC infusion at the age of 24 years. At 42 years, a melena occurred, requiring the transfusion of leucocyte- and platelet-depleted packed red blood cell (PRBC) units. A colon biopsy showed a right colon carcinoma and resulted in severe local bleeding despite PC infusions. Anti-GPIIb–IIIa isoantibodies were then detected for the first time and the patient was referred to us.

Eight IA-PA procedures were performed over a period of 12 d, with a mean duration of 8 h. A peripheral venous access was used for the first six procedures. Owing to further poor venous flow and to provide a venous access for surgery, a central venous line was inserted before procedure 7. No deleterious side-effects of the treatment were observed (Table I).

**Table I.** IA-PA procedure characteristics.

| Patients | Age (years)/ sex) | Clinical status | Number of IA-PA procedures | Duration of the treatment course (days) | Associated immunosuppressive therapy | Venous access |
|---|---|---|---|---|---|---|
| 1A | 42/M | Surgery | 8 | 12 | MP, CP (1 g) × 3, IgG × 5 | Peripheral then central |
| 1B | 44/M | Surgery | 3 | 3 | MP, IgG × 1 | Peripheral |
| 2 | 14/F | Intrahepatic bleeding | 6 | 7 | CP (400 mg) × 2, IgG × 5 | Central |
| 3A | 6/M | Cardiac tamponade | 6 | 6 | IgG × 1 | Central |
| 3B | 6/M | BMT | 6 | 6 | Azathioprine (10 mg/kg) | Central |

MP, methylprednisolone (300 mg × 3); CP, cyclophosphamide; IgG, IgG i.v. (400 mg/kg); BMT, bone marrow transplantation.

Conventional immunosuppressive therapy, including bolus i.v. methylprednisolone (300 mg × 3 on d 10, 11, 12 after the first IA procedure) and i.v. cyclophosphamide (1 g × 3 on d 2, 10 and 11), was used.

Colonic surgery was safely performed on d 13. Four PRBC were infused throughout that day, and PC were infused daily on d 13–22 (Fig 1). Rectorrhagia occurred on d 20, despite the increased number of platelets transfused, requiring the transfusion of nine additional PRBC units on d 21–27.

*Case 1B.* The same patient was referred again 2 years later in order to remove colonic polyps. Although at a lower level than previously observed, circulating anti-GPIIb–IIIa antibodies were still present (Table II). Only three IA-PA procedures were performed to restore the haemostatic activity of PC infusions. Polyp removal was uncomplicated. A peripheral venous access could be used throughout the three procedures. Bolus i.v. methylprednisolone was infused on d 4–6 (300 mg × 3).

*Case 2.* Type I GT had been diagnosed at birth in this gypsy girl in a context of consanguinity and family history with several affected relatives. Previous history included numerous bleeding episodes that required PRBC and PC transfusions, and the introduction, from age 12 years, of a continuous progestative treatment (Norethisterone®) in an attempt to prevent menorrhagia. Anti-GPIIb–IIIa isoantibodies were first detected at age 14 years, after PC transfusions for an appendectomy. The patient was again hospitalized 5 d after discharge, for right hypochondrium pain associated with dyspnoea and anaemia (Hb: 7·3 g/dl). Intrahepatic bleeding in multiple adenomas, probably related to hormonal therapy, was detected by ultrasound and computerized tomography. Numerous PC were infused but failed to stop bleeding.

She was then referred to the intensive care unit of our hospital. Her transfer was hazardous, owing to the massive haemorrhage-induced shock that required a 'G suit' to maintain blood pressure. She was intubated and ventilated.

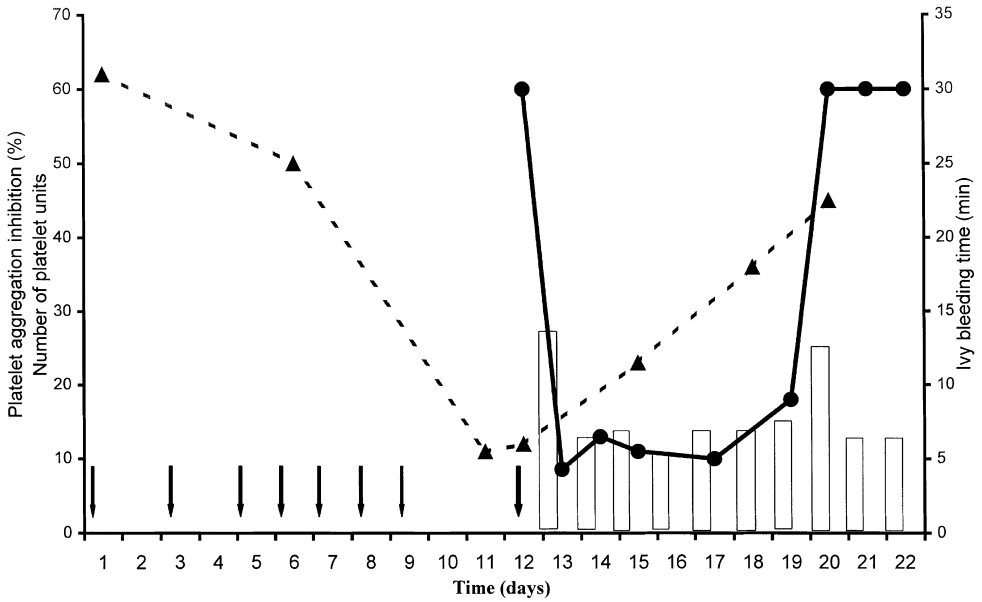


**Fig 1.** Biological evolution in patient 1A. (●) Ivy bleeding time 30 min after platelet concentrate transfusion; (▲) platelet aggregation inhibition (PAI) induced by the patient's PRP, using collagen; (↓) IA-PA procedure; (open bars) number of platelet units infused.

**Table II.** Biological results.

| Patients | IgG decrease* (%) | Anti-GPIIb–IIIa antibodies (Optical density, last positive dilution† MAIPA) | | Duration of platelet haemostatic efficacy‡ after IA-PA procedure (d) |
|---|---|---|---|---|
| | | Before IA-PA | After IA-PA | |
| 1A | 96 | + (> 3, 1/8) | + (0·9, 1/2) | 7 |
| 1B | 94 | + (0·4, 1/1) | – | 12 |
| 2 | 97 | + (0·35, 1/1) | – | 3 |
| 3A | 99 | + (0·73, 1/1) | – | 13 |
| 3B | 97 | + | – | NE |

*The overall IgG decrease after the last IA-PA procedure was expressed as percentage of the initial value.

†The last positive dilution of the sample was indicative of the antibody titre.

‡Assessed as the shortening (< 10 min) of the bleeding time 30 min after platelet transfusion.

NE, not evaluable (BMT performed on d 10).

A Hickman catheter was placed in the right internal jugular vein. After stabilization of the haemodynamic status, six IA-PA procedures were performed over a period of 7 d (Table I), combined with 2 × i.v. bolus of cyclophosphamide (d 3 and 6 after the first IA procedure) and with daily PC transfusions (4–15 PU/d).

This treatment enabled us to perform an arterial embolization of the hepatic artery through a femoral access, after PC transfusion, without any abnormal bleeding; the patient was discharged on d 20 after the first IA procedure.

*Patient 3.* A 6-year-old boy, diagnosed type I GT at birth, had experienced numerous severe bleeding episodes since early childhood, requiring frequent PRBC and PC transfusions. Anti-GPIIb–IIIa isoantibodies, first detected after the initial transfusions using the MAIPA test, had been present ever since.

*Case 3A.* A pericardial haemorrhage resulted in a cardiac tamponade, secondarily associated with a haematoma of the ileal wall and a peritoneal haemorrhage. Despite transfusion of 2–3 PU every 4 h, the clinical status worsened and the child was referred to us. IA-PA procedures were performed daily over 6 d consecutively, using a Hickman catheter placed in the right internal jugular vein (Table I). The treatment, also including 3 PU every 4 h and PRBC, made it possible to control the life-threatening haemorrhage and led to a dramatic improvement of the clinical status. Owing to the severity of the disease, and in the absence of a compatible sibling, a semicompatible bone marrow transplantation (BMT) with his mother was planned.

*Case 3B.* Three months later, the patient was referred to us again in order to remove the anti-GPIIb–IIIa isoantibodies before BMT. Six IA-PA procedures were performed (Table I) combined with daily PC transfusions; this therapy was well tolerated. Aziathioprine 10 mg/kg/week was initiated 3 weeks before BMT. Pre-BMT conditioning included antilymphocyte globulin (ALG), busulphan 16 mg/kg, cyclophosphamide 200 mg/kg, antileucocyte function-associated antigen (anti-LFA) and anti-CD2, as previously reported (Fischer, 1991). BMT was followed by a severe and prolonged (for almost 1 year) neutropenia [absolute neutrophil count (ANC) < $0{\cdot}5 \times 10^9$/l] and thrombocytopenia (< $50 \times 10^9$/l) without anaemia. A grade I graft-versus-host reaction developed, which was treated with steroids (5 mg/kg/d) and cyclosporine (5 mg/kg/d) for 2 months. Transplantation failed, as shown by the presence of autologous cells after the end of aplasia, However, the patient's clinical status improved and the anti-GPIIb–IIIa isoantibodies remained undetectable, enabling the prevention of further haemorrhages with a weekly efficient platelet transfusion over a follow-up of 4 years.

## RESULTS

### *Bleeding time*

Bleeding time, measured 30 min after PC transfusion, was dramatically shortened over a period of 7 d, 3 d and 13 d after the IA-PA procedures, in cases 1A, 2 and 3A respectively (Fig 1, Tables II and III). It returned to normal values in case 1B for 12 d (Table II).

### *Immunoglobulin levels*

Immunoglobulin levels (Table II) were dramatically decreased after IA-PA in all cases: the plasma level of IgG was decreased by 94–99% of the baseline level. IgM and IgA were also depleted, albeit to a lower extent (44–72% of the baseline level for IgM and 32–58% for IgA).

**Table III.** Biological results of patient 1A.

| | Before IA-PA | During IA-PA After 3rd IA | After IA-PA treatment course (days) After 6th IA | 1 | 6 | 9 |
|---|---|---|---|---|---|---|
| BT (min) | > 20 | | | 4·30 | 9 | > 20 |
| Anti-GPIIb–IIIa Ab (optical density, last positive dilution) | + (> 3, 1/8) | | + (0·9, 1/2) | | | + (0·95, 1/2) |
| PAI PPP/PAI PRP using collagen (%) | 64/62 | 0/50 | 0/11 | NE/11 | NE/36 | 14/45 |
| PAI PPP/PAI PRP using adrenaline (%) | 100/25 | 39/23 | 20/47 | NE/3 | NE/17 | 35/33 |

PAI PRP, platelet aggregation inhibition induced by the patient's PRP; PAI PPP, platelet aggregation inhibition induced by the patient's PPP; BT, bleeding time 30 min after platelet concentrates transfusion; NE, not evaluable.

*Anti-GPIIb–IIIa isoantibodies (Tables II and III)*

Using the MAIPA test, circulating anti-GPIIb–IIIa isoantibody levels significantly decreased soon after the first IA-PA procedure in all cases and an undetectable level was reached within 3–7 procedures, according to the initial inhibitor titre. This decrease was followed, in all assessable cases, by a secondary increase of the antibody titre, occurring, respectively, 5, 16 and 2 d after the completion of the last procedure, in patients 1A, 1B and 2, despite immunosuppressive therapy.

*Ex vivo study of platelet aggregation inhibition induced by the patient's PPP and PRP (Table III, Fig 1)*

These tests were only performed in patient 1 during the first episode. No inhibition was detected with this patient's plasma during the second hospitalization, at a time when the antibody titre, as assessed by MAIPA, had decreased; it was not detected either in the two other patients who already received platelet transfusion before admission.

1. Before IA-PA, the inhibition induced by patient's PPP was 100% and 64% of the platelet aggregation, when using adrenaline and collagen respectively. It decreased to a nadir of 20% after six IA procedures and recurred 9 d after the completion of the treatment when using adrenaline as an agonist. However, when using collagen, the inhibition became undetectable after three IA procedures and was still irrelevant (14%) 9 d after terminating the treatment.
2. Similarly, before IA-PA, the inhibition of platelet aggregation induced by patient's PRP was 62% and 25%, when using collagen and adrenaline as agonists respectively. It was still µ and 47% after the sixth IA. Two further IA procedures were thus performed, leading to an undetectable inhibitory effect as assessed using adrenaline and collagen (3% and 11% respectively). Nine days after the end of the treatment, the inhibitor had returned to 33% with adrenaline and 45% with collagen. This result correlated with an *ex vivo* prolongation of the bleeding time despite the increased number of PC transfused.

## DISCUSSION

The occurrence of transfusion-related isoantibodies in some patients with type I Glanzmann's thrombasthenia is a major concern, as it results in the loss of haemostatic efficacy of platelet transfusions. The management of such a situation, which can abruptly turn out to be life-threatening, is still poorly codified.

Desmopressin (Lethagen & Nilsson, 1992) and repeated PC infusions showed no efficacy. Plasmapheresis is a burdensome procedure with little benefit (Vivier *et al*, 1989). Recombinant Factor VIIa (rFVIIa, Novoseven®; NovoNordisk, Puteaux, France) has been shown to be effective in the treatment of haemorrhages and the prevention of surgical bleeding in such patients (Tengborn & Petruson, 1996; Wielenga *et al*, 1998; Poon *et al*, 1999, 2000; d'Oiron *et al,* 2000; Patel *et al,* 2001). However, its use may result in an overproduction of thrombin in patients with normal factor VIII or factor IX levels, and thereby in thrombosis: a severe thrombotic complication has been reported in an elderly woman with GT and anti-GPIIb–IIIa antibody who was receiving continuous infusion of high-dose rVIIa for abdominal surgery (d'Oiron *et al,* 2000; Roberts, 2001). Furthermore, rFVIIa does not achieve a bleeding time correction, even when associated with PC, and there is no reliable biological marker of its efficacy.

IA-PA has been shown to be an efficient procedure in clearing auto- or iso-IgG antibodies in patients with haemostatic defects (Kiss *et al*, 1989; Boughton *et al*, 1990; Gailani, 1992; Watt *et al*, 1992; Guillet *et al,* 2001). Our experience demonstrated that IA-PA (Excorim) was effective and well tolerated, with no deleterious side-effects, in type I GT patients and anti-GPIIb–IIIa isoantibodies on five occasions, including two life-threatening bleeding episodes and three situations with a mandating safe haemostasis in order to perform either surgery or bone-marrow transplantation.

The high efficacy of IA-PA in clearing antibodies was obvious in all cases. The subsequent restoration of the haemostatic efficacy of transfused platelets was evidenced by the shortening of bleeding time following PC infusion,

and most of all by the discontinuation and/or prevention of further bleeding. An *in vivo* beneficial effect could be demonstrated after 3–8 procedures (according to the initial antibody titre) and was sustained for 3–13 d after the last procedure (Table I). Efficacy was further demonstrated according to biological criteria: (i) An overall decrease in IgG serum level, ranging from 94% to 99% of the initial level (Table II), was observed. Of note, a decrease in IgA and IgM serum level was also observed, which confirmed the lack of complete selectivity of IA-PA in clearing IgG (Guillet *et al,* 2001). (ii) There was a specific decrease in anti-GPIIb–IIIa antibody, evidenced both by the MAIPA test and by *ex vivo* platelet aggregation tests (Tables II and III). The MAIPA test is a sensitive albeit not quantitative technique, however, the results are not available routinely, especially in emergency. Moreover, the result does not always correlate with the efficacy of platelet transfusion as shown in case 1A, where a dramatic correction of the bleeding time after platelet infusion was achieved despite the persistence of a positive MAIPA test. In this patient, the *ex vivo* aggregation tests appeared more likely to predict the *in vivo* response to platelet transfusions. This test can thus help in determining the number of procedures required for optimal antibody removal. However, owing to its poor sensitivity, it may give false-negative results when performed after massive PC transfusions, as in patients 2 and 3. In such cases, the procedures should be performed daily (including weekends) and combined with platelet infusions, in order to rapidly improve the clinical status.

The main limitations regarding IA-PA have already been reported (Guillet *et al,* 2001): (i) A large vascular access is required, and in our small series only 2/5 events could be managed using only a peripheral venous access. (ii) The restoration of PC efficacy obtained after the antibody epuration is transient, lasting 5–13 d in our experience, which may be enough to overcome life-threatening bleeding or to provide safe conditions for an invasive treatment, as shown in all the above patients. This transient efficacy is due to the enhanced synthesis stimulated by the anamnestic response elicited by normal platelet GPIIb–IIIa. Conventional immunosuppressive therapy failed to prevent a secondary anamnestic response in all patients in whom it was used. However in case 3B, heavy and prolonged immunosuppression prior to the bone marrow mismatched transplantation restored the efficacy of long-term weekly platelet transfusions, providing the patient with a near normal quality of life.

These drawbacks emphasize the need for evaluating the respective and combined use of IA-PA together with new immunosuppressive agents such as mycophenolate-mofetil (Allison & Eugui, 2000) and rFVIIa in type I GT patients who are refractory to platelet transfusions. It could be suggested that rFVIIa, the optimal doses of which remain to be defined in these patients, should be used for short periods at the onset of moderate bleeding or before minor surgery (e.g. placement of a central venous line, biopsy…) provided there is no associated risk of thrombosis and that clinical efficacy is readily demonstrated within a short period of time.

IA-PA, which has proved, in our hands, to be secure and efficient in distressing situations, should be considered as an attractive alternative in cases of severe, life-threatening continuous bleeding, or for mandatory major surgery, as it is the only treatment restoring the haemostatic clinical and biological efficacy of platelet transfusions.

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