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A novel approach using ancillary tests to guide treatment of Glanzmann thrombasthenia patients undergoing surgical procedures

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ABSTRACT

Background: Glanzmann thrombasthenia (GT) is a disorder of platelet function. Standard therapy includes platelet transfusions, which may be hampered by antiplatelet antibodies.
Aims: To assess potential correlation between bleeding and number of active platelets in GT patients undergoing surgery. Clinical peri- operative patients' hemostasis was compared with flow cytometry analysis (FC), and whole blood clot formation.
Methods: GT patients undergoing surgery were included. Blood counts, platelet activation studies, FC and rotational thromboelastography (ROTEM) were performed as ancillary tests to estimate the effectiveness of treatment.
Results: A total of 4 GT patients undergoing 5 surgeries were included. Consecutive FC analysis following platelet transfusions showed gradual decrease of donor platelets with a nadir of 3280 platelets in patients who experienced no post procedural bleeding following minor procedures. After major surgery, bleeding occurred when donor platelets decreased to 2600–4280. Decline in donor platelets was associated with reduced clot firmness as noted by ROTEM.
Conclusion: Results suggest that very low number of active donor platelets may suffice to achieve proper hemostasis in certain procedures. Our study points to the potential role of consecutive FC examinations to demonstrate the number of donor platelets as an ancillary tool for decision making in GT patients undergoing surgery.

1. Introduction

Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder of platelet function caused by mutations in the genes coding for integrin αIIbβ3 (originally termed GPIIb-IIIa): ITGA2B and ITGB3 encoding the αIIb and β3 subunits, respectively [1]. The mutations lead to quantitative or qualitative defects of integrin αIIbβ3, the fibrinogen receptor that is required for platelet aggregation. GT has been classified into three subtypes according to platelet surface expression of αIIbβ3 measured using flow cytometry (FC): Type I with absent or minimal < 5% of αIIbβ3 surface expressed. Type 2 with 5–20% of normal αIIbβ3 expression. Variant GT is a qualitative disorder with normal αIIbβ3 expression which is malfunctioning [2].

Due to impaired platelet aggregation patients present with bleeding symptoms that may be either spontaneous or trauma induced. Although the clinical manifestations of GT patients are variable, it is considered a severe bleeding disorder as > 75% of patients will require blood or platelet transfusion during their life [2]. Common symptoms of GT include mucocutaneous bleeds, epistaxis which may be life threatening, and gastrointestinal bleeding; women commonly experience menorrhagia. Less common symptoms include intracranial bleeding, hemarthrosis and haemothorax [3–5].

The standard treatment for serious bleeding events and coverage of surgical procedures is administration of platelet transfusions. Recombinant factor VII (rFVIIa) has demonstrated high hemostatic efficacy rates in surgical [6] and non-surgical bleeds [7] of GT patients. Antifibrinolytic agents may be used for the treatment of minor bleeding and as adjunct therapy for other events [8].

Platelet transfusions influence may be impeded as their half-life may be reduced due to various reasons including consumption, fever, and splenomegaly [9]. Furthermore, refractoriness to platelet transfusions due to antibodies either against the missing αIIbβ3 antigen or

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Table 1
Demographic data of patients.

Patient	Age and gender	Ethnic origin	Mutation	Platelet anti- bodies status	Previous medical history	Procedure
1	1 year F	Arab	ND	Not performed	Not significant	Diaphragmatic Hernia repair Removal of CVL (Hickman)
2	39 years F	Jewish Iraqi	ITGB3 c.2051–61 del Hom	Positive	Recurrent biliary colic	Cholecystectomy
3	19 years F	Arab	ND	Negative	Pelvic hematoma	Oophorectomy
4	9 years F	Jewish Iraqi	ITGB3 c.2051–61 del Het 11.2Kb del (IVS9aluc.2183–6) Het	Negative	Recurrent epistaxis	Teeth extraction $n = 2$

CVL - central venous catheter; F - female; ND - not detected; (despite Sanger, sequencing); NP - Not performed.

antibodies directed against MHC-class I molecules may hamper their hemostatic efficacy [10].

This manuscript describes our experiences with post platelet transfusion evaluation in several GT patients undergoing various surgical procedures. We describe clinical correlation between bleeding and number of donor platelets as evaluated by FC and rotational thromboelastrography (ROTEM). We point to the clinical correlation between number of donor platelets and the attainment of proper hemostasis.

2. Methods and patients

2.1. Patients and ethics

The Israeli National Hemophilia Center is a tertiary referral institute for about 1000 patients with severe bleeding disorders. Our center follows about 70 GT patients. Most of them (96%) have type I GT. Any GT patient undergoing surgical procedures during the last 3 years was eligible for study, once consent was provided. The study was approved by our institutional IRB in concordance with the declaration of Helsinki.

2.2. Laboratory analysis

Complete blood counts (CBC) were performed using standard technique.

Platelet aggregation (applied for patients' diagnosis): Citrated whole blood samples (9:1) was centrifuged (110 *g* 10 min) to obtain platelet rich plasma (PRP), followed by further centrifugation (1900 *g* 10 min) to obtain platelet poor plasma (PPP). Light transmission aggregometry (LTA, AggRAM, Hellena, Beaumont, TX, USA) was performed on PRP samples upon addition of various platelet agonists (final concentration: 10 μM ADP, 10 μg/mL collagen, 50 μM epinephrine and 1.5 mg/mL ristocetin). Changes in the light transmission of PRO over baseline (PPP) were recorded for 5 min and calculated as maximal aggregation.

Platelet antibodies (performed prior to surgery): Platelets from healthy donors were prepared by PRP centrifugation and washing as described previously [11]. The washed platelets were incubated with heat inactivated serum taken from the patient. Anti-platelet antibodies were detected by anti-human total Ig (IgA, IgG, IgM) – PE (phycoerythrin) conjugate (milipore, Temcuka, CA). The samples were tested by flow cytometry. Anti-αIIb β3 antibodies were detected by using either baby hamster kidney (BHK) cell expressing-αIIb β3 or only the vector (mock) as previously described [11].

Platelet FC analysis: PRP were diluted tenfold with PBS and incubated for 20 min at room temperature in the presence of monoclonal antibodies directed against αIIb: CD41-PE (Beckman Coulter, Pasadena, CA, USA) and CD61-APC against β3 (MACS Milteny Biotec Teterow, Germany). Samples were diluted fivefold with phosphate buffered saline (PBS) and analyzed by flow cytometry (FACScalibur, Beckton Dickenson. San Jose, CA).

Platelet activation studies: PRP was used for platelet activation studies with and without ADP, used as agonist. We used 2 activation markers: anti P-Selectin (CD62p) and PAC-1 monoclonal antibodies. P-Selectin is a α granule membrane protein which mediates adhesion of activated platelet and can be detected on the platelet surface only after degranulation (i.e. activation). PAC-1 monoclonal antibody is directed against fibrinogen binding site exposed by conformational change of αIIb β3 complex only in activated platelets. Donor platelets were identified by both PAC-1 and anti-P-selectin antibodies, while GT patients' platelets activation could be studied by P- Selectin only.

Thromboelastography– whole blood clot formation was evaluated by ROTEM (Pentafarm, Munich, Germany), as previously described [12].

3. Results

During the last 3 years, five surgical interventions have been conducted in 4 GT patients in our hospital. We report post transfusion FC platelet results and clinical course of our GT patients.

The demographic data of our patients is summarized in Table 1. Patients were not related to one another. All four GT patients reported fulfilled the diagnostic criteria for GT. They presented with mucocutaneous bleeding, absence of platelets aggregation in response to all physiological agonists in contrast to normal aggregation with Ristocetin. Furthermore, all patients demonstrated lack of αIIbβ3 surface expression. Molecular analysis disclosed that patient 2 was homozygous for an out of frame mutation (c.2031–2041 del, exon 13) in ITGB3 [13], and patient 4 was compound heterozygote for the same deletion and a large deletion of 11.2Kb del (IVS9aluc.2183–6) in ITGB3 [14].

Patient 1 was a 15 months old infant of Palestinian Arab origin who was transferred to our tertiary center for surgical repair of a large congenital diaphragmatic hernia (CDH). Family history was notable for consanguinity and two older siblings experienced “bleeding tendency” of undetermined etiology. Physical examination disclosed multiple skin hematomas. Patient did not require blood or platelet transfusion prior to the current hospitalization. Complete blood count, fibrinogen levels, PT and PTT were within normal range (data not shown). Platelet aggregation recorded 12% with collagen, 1% with ADP, 4% with epinephrine and normal aggregation with Ristocetin (84%), confirming GT. Baseline FC did not demonstrate any αIIbβ3 on the surface of the platelets. Prior to surgical repair of the diaphragmatic hernia, the patient was treated with platelet transfusion, tranexamic acid and a single dose of rFVIIa (1 mg). No significant bleeding was noted during the operation. Due to concerns regarding post-surgical feeding, a Hickman central venous line (CVL) was inserted. On the Seventh post-operative day (POD) a 3 g decrease of hemoglobin was noted, due to intra-abdominal post-surgical bleeding. Platelets transfusion was renewed for another 2 days and bleeding did not recur. Three weeks post CDH repair the patient was eating adequately. For removal of CVL the patient received a single platelets transfusion and regular tranexamic acid (10 mg/kg /dose, 4 times daily) was maintained. No bleeding was noted and patient did not require neither addition transfusion of blood products nor treatment with rFVIIa. For correlation between patient's bleeding symptoms and FC results see Table 2. FC analysis of patient 1 revealed 1% of donor platelets (equivalent to 4800 donor platelets) at post- operative day POD −7, when active bleeding occurred. Further consecutive FC analyses following central venous line (CVL) removal demonstrated a gradual decrease with lower trough levels of donor platelets (Table 2), however, no bleeding complications recurred.

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