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Treatment tailoring for factor V deficient patients and perioperative management using global hemostatic coagulation assays

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ABSTRACT

Introduction: Congenital factor V deficiency (FVD) is a rare bleeding disorder with an estimated incidence of 1 in 1000,000 in the general population. Since the common coagulation tests do not correlate with the bleeding tendency there is an unmet need to predict FVD patients' bleeding hazard prior to surgical interventions.
Aim: To optimize treatment prior to surgical interventions, using global coagulation assays, thrombin generation (TG) and rotating thromboelastogram (ROTEM).
Methods: Our cohort included 5 patients with FVD, 4 severe and one mild. Two of them underwent TG and ROTEM prior to surgical interventions, including ex vivo spiking assays using bypass agents and platelets spiking.
Results: All five patients exhibited prolonged PT and PTT, non-dependent on their bleeding tendency. Patient 1, who demonstrated severe bleeding phenotype, underwent surgery treated by combination of APCC (FEIBA) and platelet transfusion. Therapy was guided by global tests (TG as well as ROTEM) results. During the pre and post-operative period neither excessive bleeding nor any thrombosis was noted. In contrast, TG and ROTEM analysis of patient 4 has lead us to perform the surgery without any blood products' support. Indeed, the patient did not encounter any bleeding.
Conclusion: Global coagulation assays may be useful ancillary tools guiding treatment decisions in FVD patients undergoing surgical procedures.

1. Introduction

Congenital factor V deficiency (FVD) is a rare bleeding disorder with an estimated incidence of 1 in 1000,000 in the general population. It is 10-fold more common in west Asian countries as compared to the western world due to higher prevalence of consanguineous marriages [1,2]. The corresponding gene for factor V (FV) maps to the 1q24.2 chromosomal region and the protein is synthesized in the liver and in megakaryocytes [2]. It is inherited as an autosomal recessive disease [3]. FV has 40% identity with factor VIII (FVIII). In the coagulation cascade, FV serves as a non-enzymatic cofactor for the prothrombinase complex. Factors Va and Xa form the prothrombinase complex which catalyzes the activation of prothrombin to thrombin. In addition it plays a key role in down regulation of coagulation FVIII by enhancing the effect of protein C and therefore FV actually participates in both the pro- and anticoagulant pathways.

FVD is suspected once prolonged prothrombin time (PT) as well as activated partial thromboplastin time (aPTT) are corrected on mixing tests [4]. It is characterized by low or undetectable plasma FV levels leading to a diverse presentation ranging from asymptomatic to severe bleeding complications. Bleeding manifestations in FVD patients are variable and may include either spontaneous hemorrhages or post-traumatic and postoperative bleeding [5,6]. Approximately 25% of episodes are composed of skin and mucosal tract bleeding, however, life- threatening complications like gastrointestinal and central nervous system (CNS) hemorrhages are not common. Some women may present with recurrent miscarriages as well [7]. Severity of FVD can be classified into: mild ($\geq$ 5%), moderate (1–5%) and severe ($\leq$1%) [1]. Notably, some severe patients are asymptomatic and would be randomly diagnosed following routine blood tests [3,8].

The management of patients with FVD is challenging. Treatment is tailored towards the bleeding complications. The most common treatment for severe bleeding episodes or pre-surgical interventions is administration of fresh frozen plasma (FFP) in order to temporarily

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maintain plasma FV at a hemostatic level (20%–30%) [9]. Menorrhagia of women with FVD may be managed using oral contraceptives (OCP) [7].

Since the common coagulation tests do not correlate with the bleeding tendency (3) there is an unmet need to predict FVD patients' bleeding hazard prior to surgical interventions in order to optimize treatment, Global assays, such as thrombin generation (TG) and rotational thromboelastometry (ROTEM) have been used as complimentary tools that enable haemostatic evaluation in order to support treatment decisions [10,11].

Herein we present our tertiary center's experience. We followed and treated 5 patients with mild to severe FVD. We investigated the use of global assays as a tool for guiding intervention and therapy in various surgical and clinical circumstances in patients with FVD.

2. Materials and methods

2.1. Patients and ethics

The Israeli National Hemophilia Center is a tertiary referral institute providing comprehensive care for about 1000 patients with severe bleeding disorders. Patients with congenital FVD were identified from our institutional database and were included in our study if longitudinally followed at our center. Demographic and clinical data were collected from medical records. The study was approved by the institutional ethical committee in compliance with the Declaration of Helsinki.

2.2. Blood sampling and processing

Laboratory studies of all patients, including TG and ROTEM analysis, were performed during routine clinic visits, in a non- bleeding state, following patients' consent.

For PT, aPTT, FV, FVIII, fibrinogen, ROTEM and preparation of washed platelets, blood was taken in buffered sodium citrate tubes in a final concentration of 0.105 M. For TG analysis blood was drawn in tubes containing 0.109 M buffered citrate and 30 μg/mL (final concentration) – corn trypsin inhibitor (CTI) (formulated by HTI Haematologic Technologies Inc., Essex Junction, Vermont, USA). Platelet-poor plasma (PPP) was obtained at room temperature by centrifugation of blood at 2000 g for10 min followed by centrifugation at 14,000 g for 5 min. Platelet-rich plasma (PRP) was prepared by centrifugation of blood at 130 g for 10 min and adjustment of the platelet count to 150,000/μL with autologous PPP. For the preparation of washed platelets, washing buffer (140 mM NaCl, 2.9 mM KCl, 0.36 mM NaH2PO4, 5 mM dextrose, 0.2 μg/mL PGE1 and 1.0 U/mL apyrase, pH 6.5) was added to the PRP (1:1 vol/vol). The platelets were then isolated by centrifugation at 800 g for 10 min at room temperature and re-suspended in 1 mL washing buffer. Following a second centrifugation at 800 g for 10 min, platelets were suspended in Tyrode-HEPES buffer (140 mM NaCl, 2.9 mM KCl, 0.36 mM NaH2PO4, 5 mM dextrose, 10 mM HEPES, pH 7.4). Platelet concentration was adjusted to 150,000/μL with the tested PPP.

2.3. Laboratory analysis

Coagulation assays: PT, aPTT, Fibrinogen, FV and FVIII, were performed using ACL-TOP-autoanalyzer (Instrumental Laboratories, Bedford, MA, USA), and HemosIL reagents: RecombiPlasTin 2G, SynthASiL, Fibrinogen C, FVIII deficient plasma, FV deficient plasma, Factor diluent, Calibration plasma, Normal control assayed plasma and Special test control level 2 (Instrumental Laboratories, a Werfen company), according to the manufacture instructions. Testing for the absence of an inhibitor anti FV or FVIII was examined in all patients according to Triplett & Harms [12].

2.4. Calibrated automated thrombin generation assays

TG was measured in PPP or PRP using the calibrated automated thrombinography (CAT) method [13]. Twenty microliters PPP-Reagent low or PRP reagent (Stago, Gennevilliers, France)were added to 80 μL PPP or PRP, respectively and placed in a round-bottom, 96-well plates. In the TG spiking assays recombinant activate factor VII (rFVIIa) (NovoNordisk, Bagsvaerd, Denmark) or APCC (activated prothrombin complex concentrate) namely factor VIII inhibitor bypassing activity (FEIBA) (Baxter, Vienna, Austria) were added to the plasma. TG was initiated by adding 20 μL of fluorogenic substrate/CaCl2 buffer (FluCakit, Stago). Fluorescence was measured with a fluorometer (Fluoroskan Ascent, Lab System, Helsinki, Finland) with an excitation filter at 390 nm and an emission filter at 460 nm. The results were displayed as plots and derived parameters (i.e., endogenous thrombin potential [ETP], (nM × min) and peak height (nM)) were calculated by dedicated computer software attached to the fluorometer (version 3.0.0.29 Thrombinoscope-BV).

2.5. ROTEM

Whole blood clot formation was evaluated by thromboelastography, using ROTEM-delta instrument (ROTEM®, Instrumental Laboratories, a Werfen company). ROTEM was performed using three systems for coagulation activation: $CaCl_2$ [Star-tem ®], tissue factor [Ex-tem®] elagic acid [In-tem®] measuring CT – clotting time [sec], MCF- maximum clot firmness [mm] and alpha angle slope [°].

3. Results

3.1. Patients

Five patients were identified with FVD and followed, of which 4 had severe FVD. Their demographic characteristics are described in Table 1.

3.1.1. Patient 1

Patient 1, a familial case (sibling of patient 2),was diagnosed at the age of 6 years due to rectal bleeding for which she was treated with FFP and cryopercipitate. Following FFP administration she developed an anaphylactic reaction which reoccurred despite pre-medication and was life threatening therefore she did not receive plasma onwards. The issue was discussed with the patient who refused any further

Table 1
Demographic characteristics.

Patient	Gender	Age at diagnosis	Origin	Family history of FVD	Bleeding tendency	Co-morbidities
1[a]	F	6 years	Ukraine - Jewish	Yes[a]	Severe	
2[a]	M	4.5 years	Ukraine - Jewish	Yes[a]	No	s/p TGA repair at 11 days, MTHFR C677T-Hom.
3	F	59 years	Yamen - Jewish	No	Severe	Prothrombin A20210T Het.
4	F	52 years	Polish - Jewish	No	No	Hypercholestrolemia Smoking
5	M	8 days	Kavkaz -Jewish	No	No, except at circumcision	

[a] Sibling.