Prothrombin Time and International Normalized Ratio as Predictors of Factor VII Coagulation Activity in Pediatric Patients

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ABSTRACT **Background:** Factor VII (FVII) deficiency is characterized by normal activated partial thromboplastin time (aPTT) and prolonged prothrombin time (PT) values. It is diagnosed by determining protein level and coagulation activity (FVII:C). FVII:C measurements are expensive and time consuming.

Objectives: To analyze correlations between PT, international normalized ratio (INR), and FVII:C in pediatric patients before otolaryngology surgery and to establish alternative methods for identifying FVII deficiency.

Methods: FVII:C data were collected from 96 patients with normal aPTT and prolonged PT values during preoperative otolaryngology surgery coagulation workup between 2016 and 2020. We compared demographic and clinical parameters using Spearman correlation coefficient and receiver operating characteristic (ROC) curve analysis to determine the accuracy of PT and INR values to predict FVII deficiency.

Results: The median values of PT, INR and FVII:C were 13.5 seconds, 1.14, and 67.5%, respectively. In total, 65 participants (67.7%) displayed normal FVII:C compared to 31 (32.3%) with decreased FVII:C. A statistically significant negative correlation was observed between FVII:C and PT values and between FVII:C and INR. Despite statistically significant ROC of 0.653 for PT (*P*-value = 0.017, 95% confidence interval [95%CI] 0.529–0.776) and 0.669 for INR (*P*-value = 0.08, 95%CI 0.551–0.788), we were unable to determine an optimal cutoff point to predict FVII:C deficiency with high sensitivity and high specificity.

Conclusions: We could not identify a PT or INR threshold to best predict clinically relevant FVII:C levels. When PT is abnormal, determining FVII:C protein levels is needed for diagnosing FVII deficiency and considering surgical prophylactic treatment.

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KEY WORDS: Factor VII (FVII) deficiency, prolonged prothrombin time (PT), international normalized ratio (INR), preoperative coagulation screening

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Coagulation Factor VII (FVII) is a zymogen of a vitamin K-dependent serine protease. It is synthesized primarily by the liver as a single chain 50 kDa protein that is protolyzed into an activate form called FVIIa [1]. Through its interaction with tissue factor (TF), FVIIa initiates the extrinsic pathway [2] ultimately inducing the formation of fibrin [3]. The FVII gene [4], is inherited in an autosomal recessive manner with variable expression and high penetrance [5]. Factor VII deficiency is a rare coagulation disorder that can be classified into two subtypes: type I (quantitative defects, with low FVII protein levels [FVII:Ag] and subsequently decreased FVII coagulation activity [FVII:C]) and type II (qualitative defects, with lower range-normal FVII:Ag and decreased FVII:C). Type II FVII deficiency is usually characterized by an FVII:C below 70% of normal levels, even though clinically relevant bleeding events typically manifest when FVII:C is < 30% (considered the clinical manifestation threshold) [6]. FVII deficiency presents a wide range of clinical manifestations from asymptomatic to potentially life-threatening hemorrhages [7]. Complete absence of FVII:C is usually incompatible with life, and individuals die shortly after birth due to severe hemorrhage [8].

The diagnosis of FVII deficiency is usually established after recurrent bleeding episodes requiring clinical evaluation, during screening of family members in cases of familial coagulation disorders, or incidentally, as part of the preoperative workup. Routine laboratory coagulation tests include prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and platelet count. Patients with normal aPTT and prolonged PT values are suspected to have FVII deficiency and require further testing to confirm diagnosis and determine subtype. This assessment includes FVII plasma levels (FVII:Ag), protein activity (FVII:C), and molecular analysis of the FVII gene. The degree of FVII coagulation activity is sensitive to the type of TF used in assay (human, animal, or recombinant source) and should consequently be addressed with caution [9].

The prevalence of FVII deficiency can vary within different populations and is generally estimated to be approximately 1:300,000 to 1:500,000. This estimate is likely undervalued since asymptomatic patients with decreased FVII:C might not be screened [10]. Interestingly, there is an increased prevalence

of FVII deficiency in highly homogenous populations that practice consanguineous marriage, such as in Israel [11] and Iran, in contrast to Italy and the United Kingdom [12·13]. Over 130 different FVII variations have been previously described, with many of them indicating a founding effect such as the Ala244Val variant seen among Iranian and Moroccan Jews in Israel [14,15]. DNA variations can affect gene expression or protein activity and result in FVII deficiency that does not always correlate with disease severity [16]. This lack of correlation poses a significant challenge to establish guidelines for initiating prophylactic therapy in a preoperative setting. Currently, assessing the bleeding risk includes a combination of patient and family bleeding history, the surgery type, and the level of FVII:C [17]. A low bleeding risk is established in the absence of either a personal or familial history of bleeding with FVII:C > 20%. In contrast, FVII:C < 20% and personal or familial history of bleeding is considered a high bleeding risk [18]. In our institute, prophylactic therapy of tranexamic acid (10–15 mg/kg 3 times a day) is given prior to a tonsillectomy and adenoidectomy (T&A) surgery because it is performed in anatomic areas with a tendency to bleed. This treatment is administered to pediatric patients based solely on decreased FVII:C levels (< 60%) because these young patients may not have yet experienced a hemostatic challenge.

Determining FVII:C is expensive and time consuming; therefore, we established an alternative method for identifying significant FVII deficiency in patients and decided when prophylactic treatment should be initiated. Since prolonged PT and INR values are routinely obtained and are the first step in establishing a FVII deficiency diagnosis, we were interested in determining whether these values alone were enough to diagnose patients with FVII deficiency.

PATIENTS AND METHODS

STUDY OVERVIEW

We conducted a single-center retrospective study of 96 pediatric patients (≤ 18 years) referred to the pediatric hemato-oncology department at the Sanz Medical Center–Laniado Hospital, Netanya, Israel, between 2016 and 2020 for evaluation of prolonged PT and/or prolonged INR and normal aPTT values prior to a T&A surgery. Children with any background illness or the regular use of prescription medication that may affect blood clotting were excluded. We reviewed patient electronic records/written charts for demographic characteristics, underlying diseases, and laboratory parameters (PT, INR, and FVII:C) at admission to Sanz Medical Center. All blood tests were performed in the same laboratory utilizing a standardized based value index that remained constant throughout this study. The index included PT and INR values that ranged between 9.4–12.8 seconds, 0.86–1.1, respectively. FVII:C

Table 1. Patient characteristics according to FVII deficiency status

Parameter			FVII deficiency (FVII:C < 60%)		*P*-value
			Yes (n=31, 32.3%)	No (n=65, 67.7%)	
Age in years, median (range)		10 (1.0–18)	10 (1.2–18.0)	10 (1.0–18.0)	0.897
Sex, n (%)	Male	58 (60.4%)	19 (61.3%)	39,60.0%)	0.904
	Female	38 (39.6%)	12 (38.7%)	26 (40.0%)	
Ethnicity, n (%)	Sephardic	46 (47.9%)	15 (48.4%)	31 (47.7%)	0.756
	Ashkenazi	32 (33.3%)	11 (35.5%)	21 (32.3%)	
	Mixed	11 (11.5%)	4 (12.9%)	7 (10.8%)	
	Arabs	7 (7.3%)	1 (3.2%)	6 (9.2%)	
PT in seconds (median, range)		13.5 (10.9–18.9)	13.7 (11.6–16.4)	13.4 (10.9–18.9	**0.017**
INR in seconds (median, range)		1.14 (0.9–1.6)	1.17 (1.1–1.4)	1.14 (0.9–1.6)	**0.008**
FVII:C (median, range)		67.5% (31–140)	46% (31–59)	76% (60–140)	**< 0.001**

Normal values: FVII:C > 60%, INR = 0.86–1.1, PT = 9.4–12.8 seconds

FVII = Factor VII, FVII:C = Factor VII coagulation activity, INR = international normalized ratio, PT = prothrombin time

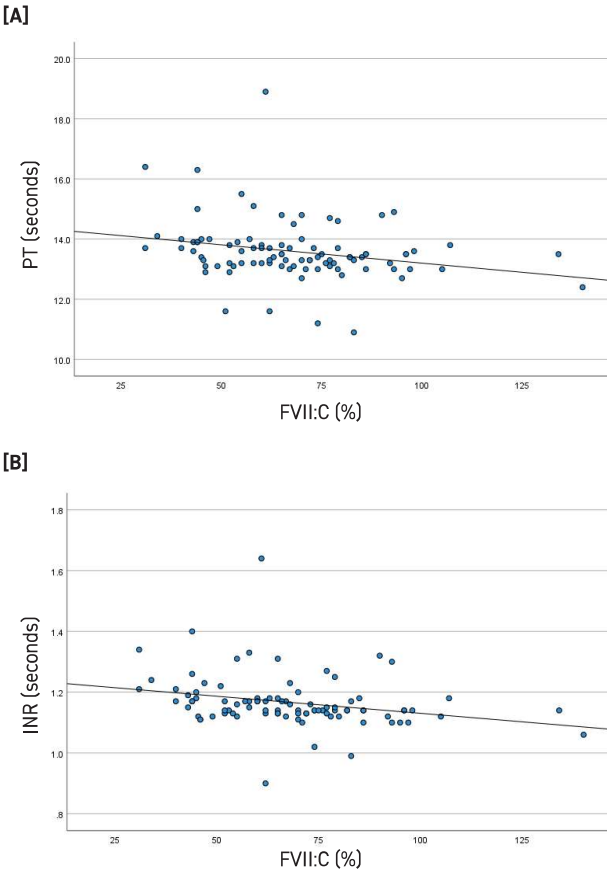
Bold signifies significance

levels ranged between 60% and 171%. The parameters were similar during all the years of this study. Approval for this study was granted by the Helsinki Committee of Sanz Medical Center–Laniado Hospital. All procedures were performed according to good clinical practice and the Israeli Ministry of Health regulations for the conduct of clinical studies.

FVII ANALYSIS

We collected 3.5 ml of whole blood into vacuum tubes (Greiner, Kremsmunster, Austria) containing 1/10 volume of 3.2% trisodium citrate. The samples were centrifuged for 8 minutes at room temperature at 2500 grams, and the plasma was utilized for analysis within 2 hours. FVII activity was measured by the one-stage clotting assay based on aPTT, and factor VII-deficient plasma (Dade, Siemens, Marburg, Germany) was measured on a CS2500 instrument (Sysmex, Kobe, Japan) using a recombinant human tissue factor (rHTF, Dade, "Innovin").

Figure 1. Scatter plots showing the correlation between **[A]** FVII:C to PT (r^2 = 0.059) and **[B]** FVII:C to INR (r^2 = 0.069)



FVII = Factor VII, FVII:C = Factor VII coagulation activity, INR = international normalized ratio, PT = prothrombin time

STATISTICAL ANALYSIS

Data were tested for normal distribution using the Kolmogorov–Smirnov test. Categorical data were reported as frequency (n) and percent (%) and numerical data as mean ± standard deviation (SD) or median (range). The correlation of PT and INR with FVII deficiency was investigated using Spearman's correlation coefficient (rs). The comparison of the results between the groups was done with Mann-Whitney rank sum test or with Kruskal-Wallis one way analysis of variance on ranks for more than two groups for the cases in which the normality test failed. In addition, area under curve (AUC) and 95% confidence interval (95%CI) of the receiver operator characteristics (ROC) curve were compared to analyze the accuracy of the PT and INR value to predict FVII deficiency. The optimal statistical significance was set as a two-tailed *P*-value < 0.05. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 28 (SPSS, IBM Corp, Armonk, NY, USA).

RESULTS

Over the 4-year study period, 96 pediatric patients (58 males, 60.4%), median age range of 10 (1.0–18) years, were included in this study [Table 1]. Most patients were Sephardic Jews (n=46, 47.9%), followed by Ashkenazi Jews (n=32, 33.3%), mixed ethnicity (n=11, 11.5%), and Arabs (n=7, 7.3%). The median PT of the cohort was 13.5 (10.9–18.9) seconds, and the median INR was 1.14 (0.9–1.6), both slightly prolonged. The median FVII:C of the cohort was 67.5% (31–140%), while none of the patients presented with severe FVII:C deficiency (< 20%).

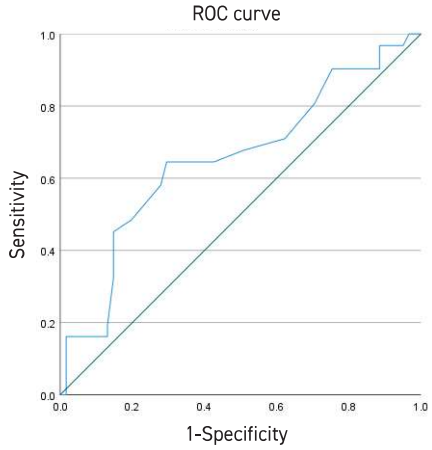
Thirty-one patients (32.3%) were diagnosed with FVII deficiency, with a statistically significant lower median FVII:C of 46% compared to FVII:C of 76% in patients without FVII deficiency (*P* < 0.001). In addition, both PT and INR values were significantly prolonged in the FVII deficiency group compared to patients with normal FVII (13.7 vs. 13.4 seconds [*P* = 0.017] and 1.17 vs. 1.14 [*P* = 0.008], respectively). No significant statistical differences were found comparing age, sex, and ethnicity between these two groups.

To analyze the correlation between PT and INR values and FVII:C, we used Spearman's correlation coefficient analysis. Scatter plots demonstrating the correlation are presented in Figure 1. A negatively significant statistical correlation was found between FVII:C and PT value (correlation coefficient -0.303, *P*-value = 0.003) and between FVII:C and INR value (correlation coefficient -0.366, *P*-value < 0.001).

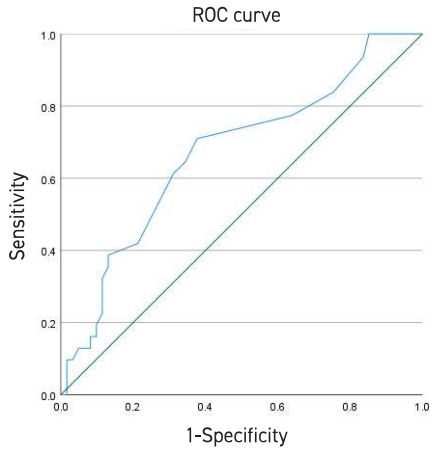
In addition, we performed a ROC curve analysis to evaluate the ability of PT and INR to predict FVII deficiency [Figure 2]. The AUC was 0.653 (*P*-value = 0.017, 95%CI 0.529–0.776) for PT and 0.669 (*P*-value = 0.08, 95%CI 0.551–0.788) for INR. Unfortunately, we could not find an optimal cutoff point to predict FVII deficiency with PT or INR values because none of the

Figure 2. Receiver operating characteristic (ROC) curve analysis

[A] FVII:C status and PT value



[B] FVII:C status and INR value



FVII:C = Factor VII coagulation activity,
INR = international normalized ratio, PT = prothrombin time

points included both high sensitivity and high specificity.

Regarding the otolaryngology procedures, all patients with FVII deficiency were treated with prophylactic tranexamic acid according to our institute's protocol and none of the patients presented with acute bleeding that required surgical intervention or blood transfusion.

DISCUSSION

We reported data of 96 pediatric patients undergoing FVII:C evaluation according to prolonged PT or INR before an otolaryngology procedure. Despite the significant statistical difference in the FVII:C, PT, and INR values between patients with FVII deficiency compared to patients with normal FVII and the significant correlation between PT with FVII:C values and INR with FVII:C values using Spearman's coefficient analysis, we were unable to determine a specific cutoff for predicting FVII:C deficiency using PT or INR. This interpretation is due to our ROC analysis, which clearly demonstrated that very prolonged PT and INR values provided high sensitivity but remarkable low specificity in predicting FVII deficiency among our cohort.

Management of coagulation hemostasis in the surgical setting is crucial since inadequate bleeding control is associated with increased complications and mortality. Identifying patients at increased risk will help clinicians provide the best surgical management and inform parents about the increased risk of bleeding postoperatively. However, FVII deficiency has a broad spectrum of bleeding symptoms, and coagulation tests are inefficient in predicting the bleeding risk in individual patients. Therefore, interpreting the coagulation tests together with family and personal bleeding history is necessary. Despite its importance, there are currently no standard guidelines on routine hematological evaluation prior to otolaryngology surgeries. Many institutions rely on positive medical and family history of bleeding disorders as precedent for further evaluation. An *otolaryngology* surgery may represent the first significant bleeding challenge for otherwise healthy children and is an opportunity to recognize and treat previously unknown bleeding disorders. We determine whether in cases where FVII deficiency is suspected, PT and INR values could be used instead of FVII:C to initiate prophylactic treatment before surgery. FVII:C measurements are complex and time-consuming. Therefore, these measurements would simplify the current protocols available for initiating prophylactic therapy in FVII deficiency patients. Although INR was originally developed to monitor vitamin K antagonists and not for the evaluation of bleeding disorders, this value has been used in previous studies to detect blood abnormalities [19]. Despite observing a negative correlation between PT and INR values to FVII:C, we could not identify a threshold for low FVII:C. Furthermore, we observed over 67% of patients with prolonged PT yet normal FVII:C, expressed by very low sensitivity in the ROC analysis. Since FVII levels are affected not only by genetic variations but also by environmental factors such as obesity, underlying disorders, vitamin K deficiency, and liver disorders, but rarely by inhibitors [20], this finding warrants further investigation that is out of the scope of this study and includes repeat testing of FVII levels on more than one occasion to rule out any transient variations.

In addition, our results support the hypothesis, which suggests environmental factors and/or other genetic components could play a role in the modulation of both the function of the clotting process and the penetrance of FVII deficiency. As expected, we observed an ethnic effect with a higher prevalence of Sephardic Jews (Northern Africa and Asian descent) presenting with FVII deficiency, but without statistical significance. Earlier reports

have established that the predominant mutation causing FVII deficiency in Israel is the Ala244Val variant, which can negatively affect FVII:C up to 75% [15]. This mutation was confined mainly to Iranian and Moroccan Jewish patients (Sephardic Jews) and could partly explain the ethnic effect we observed.

Our study has several limitations. First, as a retrospective study, there is a potential bias regarding the medical data reported in patient medical records. In addition, our cohort was relatively small, which may be the reason we did not reach a clear cutoff for defining FVII deficiency with only PT or INR values. Further studies with larger cohorts will need to be done to keep investigating this hypothesis.

CONCLUSIONS

Diagnosis and treatment of occult coagulopathy in the preoperative setting remains challenging. Despite the general rarity of FVII deficiency, its potential role in surgical complications makes it a relevant problem for pediatric clinicians, especially in Israel, where there is an increased prevalence. In conjunction with our results, we conclude that FVII:C testing is still needed to exclude FVII deficiency when PT or INR is prolonged.

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I am patient with stupidity but not with those who are proud of it.
Edith Sitwell (1887–1964), British poet and critic

Capsule

Taking a toll on the aorta

Calcification of the aortic valve is a major cause of aging associated cardiovascular disease. In this condition, fibroblasts residing within the aortic valve acquire a bone forming phenotype and deposit calcium, which damages the valve. By studying samples from human aortic valves, as well as those from mice and zebrafish, **Gollmann-Tepeköylü** and colleagues identified a pathway involving toll-like receptor 3 and interferon signaling that contributes to aortic calcification. In young animals, this pathway is necessary for normal bone formation, but it acquires a pathogenic activity later in life and may be amenable to pharmacological intervention.

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