THROMBOELASTOMETRY IN PEOPLE WITH SEVERE HEMOPHILIA UNDER LOW DOSES PROPHYLAXIS

THROMBOELASTOMETRIE CHEZ LES HEMOPHILES SEVERES EN PROPHYLAXIE FAIBLES DOSES

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Abstract

There is a heterogeneity in bleeding phenotype within patients with severe haemophilia. The study aim's were to assess the Rotational thromboelastometry ROTEM® in people with severe hemophilia under low prophylactic dose and to correlate with phenotype. Severe hemophilia patients under low dose of prophylaxis (10 hemophilia A and 2 hemophilia B) were included. The clinical features were reviewed. Chronometric FVIII or FIX activity and ROTEM® were performed at T0 and after infusion T30 minutes. Eight patients have bleeding symptoms. There was no association between clotting factor level at T0 and T30 and bleeding events. The comparison of the different ROTEM® parameters showed a significant difference between T0 and T30 only for the clotting time CT;p=0.004. We didn't demonstrate an association between ROTEM® parameter and the bleeding events. Although the absence of correlation between the patient's phenotype and ROTEM® parameters, further studies are needed to predict bleeding phenotype.

Key - words : Haemophilia ;Rotational thromboelastometry ; Bleeding ; Prophylaxis

Résumé

La thromboélastométrie rotative ROTEM® semble être utile pour évaluer le phénotype hémorragique des hémophiles sévères. Les objectifs de l'étude étaient de réaliser la thromboélastométrie chez les hémophiles sévères sous faibles doses de prophylaxie et de rechercher une corrélation avec le phénotype. Douze hémophiles (10 hémophiles A et 2 hémophiles B) sous faibles doses de prophylaxie ont été inclus prospectivement. Les données cliniques ont été recueillies. L'activité chronométrique du FVIII ou IX et la ROTEM® ont été réalisées à T0 et T30 après l'infusion de la dose prophylactique. Huit patients ont présenté des symptômes hémorragiques. Une différence statistiquement significative entre T0 et T30 a été notée uniquement pour le paramètre temps de coagulation (CT) ; p=0,004. Il n'a pas été trouvé d'association entre les paramètres de la ROTEM® et les événements hémorragiques. Des études prospectives multicentriques sont nécessaires pour prédire le phénotype hémorragique chez les hémophiles sévères.

Mots - clés : Hémophilie ; Thromboélastométrie rotative ; Saignement ;Prophylaxie

ملخص

يبدو بأن قياس الخثرة الدوراني #روتيم# مفيدا لتقييم النمط الظاهري لنزيف المصابين بالهيموفيليا الشديدة.
كانت أهداف هته الدراسة إجراء قياس الخثرة في مرض الهيموفيليا الحاد تحت جرعات منخفضة من العلاج الوقائي و البحث عن ارتباط مع النمط الظاهري.
تم تضمين 12 حالة منها 10 من صنف أ و2 من صنف ب و ذلك لتلقي جرعات علاج وقائي مستقبليا.
تم جمع البيانات السريرية و تم إجراء النشاط الزمني لعاملي رقم 8 و رقم 9 في الدم و روتيم في ت0 و ت30 لضخ الجرعة الدوائية و ذلك باعتماد وقت التخثر.
ظهرت على 8 مرضى أعراض النزيف. لوحظ وجود فرق معتد به إحصائيا بين ت0 و ت30 فقط لتأشير وقت التخثر مع بي أقل من 0.004.
لم نجد ارتباط بين معلمات روتيم و الأحداث النزيفية.
تبقى حصول دراسات استشرافية مستقبلية هامة لربط النمط النزيفي لدى مرضى الهيموفيليا من النوع الحاد.

الكلمات المفاتيح: الهيموفيليا ; قياس الخثرة الدوارة ; النزيف ;الوقاية

INTRODUCTION

Hemophilia is a rare inherited bleeding disorder due to a deficiency in factor FVIII (hemophilia A) or factor FIX (hemophilia B). It's characterized by a clinical phenotype heterogeneity. Coagulation factor assays useful in the severity classification have been shown to be limited in managing clinical phenotype. Prophylaxis with low doses ~~in our country~~ is a therapeutic option in our socio-economic conditions [1,2]. In order to assess the impact of this treatment, we opted to study the thromboelastometry in people with severe hemophilia before and after treatment, and to correlate with FVIII or FIX activity levels and the clinical phenotype.

PATIENTS AND METHODS

The study was prospective including 12 people with severe hemophilia under low doses of prophylaxis followed at the hemophilia treatment center after giving their oral consent. A collection of clinical and biological data was carried out from medical records. Two sodium citrate blood samples were collected before the infusion of the prophylactic dose (T0) and 30 minutes (T30) after. The one stage partial thromboplastin time based assay was performed on the STA Compact® automated system to determine FVIII or FIX activity at T0 and T30. A screening for FVIII or FIX inhibitors was done by the Bethesda method on the T0 sample and completed by a titration if the result was positive. Real-time blood clot formation was recorded by rotation thromboelastometry (ROTEM®, Pentapharm GmbH, Munich, Germany) at T0 and T30. A quality control was tested for the validation of the reagents using the Rotrol N ® and Rotrol P ® kit. The ROTEM® was performed within 30 minutes after the venous blood sample at T0 and T30. The test was carried out by the same operator for a total duration of 60 minutes. Citrated blood samples were preheated to 37 ° C and added to the reagents supplied by the manufacturer using the electronic pipette connected to the analyzer. We studied the INTEM profile.
The parameters analyzed in our study were:

- **Clotting Time (CT) :** expressed in seconds.

- **Clot Formation Time (CFT)** : expressed in seconds. this is the time between CT and obtaining a clot firmness of 20 millimeters (mm).

- **Maximum Clot Formation. (MCF)** : is the maximum amplitude of the clot firmness during the execution time expressed in mm.

- **Angle α:** is the angle between the baseline and the tangent to the curve at the point of 2 mm; expressed in degrees.

Statistical analysis was done by SPSS version 21 software using student's T test for comparison of means, Mann-Whitney test for association analysis between clinical phenotype and ROTEM®'s parameters as well than the factor rate. The threshold was set at 0.05.

RESULTS

Twelve patients with severe hemophilia on low-dose prophylaxis had participated in the study: 10 hemophilia A and 2 hemophilia B. One hemophilia B patient is carrying FIX inhibitor (28.8 Bethesda Unit) with clinical response to FIX subtitution. Eight patients on prophylaxis have bleeding symptoms during the year of the study. (Table 1)

Measurement of FVIII / FIX level : Except the case of hemophilia B with inhibitor (P12), the average factor level found at T0 in our study was ≥ 1%. There was non statistical significant association between the level of clotting factor at T0 and T30 and bleeding event.

Inhibitor screening : It was negative for all patients except patient P12 who is known to have FIX inhibitor before inclusion.

Thromboelastometry profiles : Table 2 shows the results of the different parameters of ROTEM® before and after the infusion of the prophylactic dose. The comparison of the means of the different parameters analyzed showed a statistically significant difference between T0 and T30 only for the CT parameter ($p = 0.004$). For the hemophilia patient B with inhibitor, the parameters of ROTEM ® were undetectable.

Correlation between the values of ROTEM® and the presence of the bleeding symptoms: The median CT in patients with and without bleeding events was respectively 621 sec (288-1117) and 607sec (324-1638) at T0 and 252 sec (192- 361) and 239 sec (222-253) at T30. No association was found between CT and the occurrence of bleeding events during the year at T0 ($p = 0.2$) and T30 ($p = 0.8$). No association was found between the other parameters of ROTEM® (CFT, MCF and angle α) at T0 and T30 and the appearance of a hemorrhagic syndrome during the year.

Table I : Clinical and biological features

Patient	Age	Bleeding event	dose in UI/Kg	Injection number/ week	FVIII/FIX Level T0	FVIII /FIX Level T30
P1	24	+	23.2	once	1	20
P2	10	+	20	twice	1	21
P3	11	+	15.1	thrice	2	21
P4	14	+	10.4	twice	1	15
P5	12	+	15.6	thrice	1	14
P6	21	+	25.4	once	4	36
P7	14	+	15.8	twice	1	12
P8	30	-	16.1	twice	1	21
P9	23	-	10.4	twice	1	20
P10	10	-	10	twice	2	11
P11*	11	-	8	twice	1	12
P12*	14	+	13.1	twice	<1	<1

* Patient with Hemophilia B

Table II : Parameters of ROTEM® before and after the prophylactic dose

	Before Infusion				After infusion				p
	mean	**SD**	**Min**	**Max**	**mean**	**SD**	**Min**	**Max**	
CT sec	692,9	393,7	288	1638	251,2	42,86	192	361	0.004
CFT sec	419,2	473,9	128	1795	175	65,5	109	283	0.2
MCF Mm	53	10,2	27	66	52,8	5,3	43	63	0.4
Angle α	52,8	15,7	29	76	63,4	8,3	46	72	0.2

SD : Standard deviation /Min : Minimum /Max : Maximum/CT :Clotting Time /CFT :Clot Formation Time /MCF :Maximum Clot Formation

DISCUSSION

In this study, the ROTEM® in people with severe hemophilia on low doses of prophylaxis was analyzed looking for a correlation with the presence of bleeding events in the year of the study. Except the case of hemophilia B with inhibitor, the mean factor level found at T0 in our series was ≥ 1%. The value found corresponds to the factor level in moderate hemophilia (1-5%). Thus, the prophylactic protocol applied would be adequate and it achieved one of its objectives of maintaining the factor level ≥ 1% [3, 4]. The threshold of 1% in prophylaxis regimen is not the optimal aim of prophylaxis nowadays. Nevertheless, low doses prophylaxis is an alternative to introduce prophylaxis in countries with limited resources.

The correlation between the level of FVIII or FIX in patients on prophylaxis and the appearance of bleeding events is variable from one individual to another and remains controversial according to the data in the literature. In the Collins et al study including 143 severe hemophilia A patients under high dose prophylaxis, a statistically significant association was found between the time spent with an FVIII rate <1% and the frequency of the bleeding events (p <0.002) [5]. However, according to Ahnstrom Jet al, there is no association between the level of FVIII or FIX and the incidence of hemorrhagic syndrome [6]. In our series, there was no association between the factor levels at T0 and T30 and the occurrence of bleeding events during the year.

The means of the parameters obtained from ROTEM® are longer than the normal reference values. A statistically significant difference between the CT values before and after prophylaxis was found. An improvement in the CFT parameters and the angle α was observed, but without reaching the significance level. The value of MCF obtained is practically the same before and after prophylaxis. This is consistent with the results found by Frank Driessler et al [7].

The hemophilia B patient with inhibitor did not develop the ROTEM® tracing. Thromboelastometry parameters were not detected. This is compatible with the results of a Japanese study carried out on hemophilia patients with inhibitors [8]. However, these parameters were detected in the study of M. Shima et al [9]. The development of antibodies is one of the most complications reducing the effectiveness of treatment and making the management of hemorrhagic syndrome more difficult [10].

In our study, the ROTEM ® traces obtained were variable in patients of the same severity. These results are consistent with other studies. Many influencing factors are reported by the literature such as the existence of polymorphism at the level of factor V Leiden and the prothrombin gene G20210A and the rate of monocytes as an endogenous source of tissue factor [11,12]. It would therefore be necessary to complete with a thrombophilia test for the patients included in our serie.

Several authors have described the advantage of ROTEM® and of thromboelastography TEG® in the evaluation of the hemophilia phenotype, in particular for better adaptation of the prophylactic dose. The interest of these techniques has also been reported in the monitoring of hemophilia with inhibitors under bypass treatment (Novoseven and Feiba) [13]. In the series of Driessler F et al; a statistically significant correlation was noted between the CT after injection of the prophylactic dose and the frequency of the occurrence of the hemorrhagic syndrome (p = 0.003) [7]. However, this correlation was not found at T0. Also according to the study by Chitur M. et al, by performing TEG® in 20 severe hemophilia patients of variable phenotype, a significant correlation between the maximum rate of thrombin generation (MTG) and the bleeding tendency was noted [11]. However; in our study, no statistically significant correlation was found between the ROTEM® parameters (CT, CFT, MCF and angle α) and the bleeding events in pre (T0) and post (T30) prophylactic dose injection. In the study of Zetterberg et al in 2017, including 21 severe hemophilia patients on prophylaxis, no correlation was observed between the ROTEM® parameters, the thrombin generation test (TGT) and FVIIIc on the one hand and the frequency of bleeding events on the other hand [14]. The results of studies concerning the analysis of the correlations between the ROTEM®, TEG® tests and bleeding phenotype are variable in the literature. This could be explained by the heterogeneity of the population studied (number included, type and severity of hemophilia and presence or absence of inhibitor), regimens treatment, techniques used, etc.

Several difficulties were observed during the study. The number of patients included was low due to the difficulties in recruiting patients who are in the majority of cases young children. It was also

impossible to perform the ROTEM® for more several samples at different times, since the majority of patients live far from the hospital and are at low socioeconomic level. In fact, it should be interesting to determine for each patient the time spent with a level of the deficient factor <1% which seems to be more associated with the severe clinical phenotype. The population studied was heterogeneous, combining both hemophiliacs A and B, (with and without inhibitor). In addition, the ROTEM® technique used in hemophilia patients has some particularities and it is being standardized by the TEG / ROTEM working group of the international society of hemostasis and thrombosis [15]. It is recommended to use the own reference values in each laboratory with internal quality controls.

Our results are preliminary to study the contribution of thromboelastometry in the management of hemophilia in our center. In perspective, a large multicenter study is recommended to analyze the usefulness of ROTEM® in the evaluation of phenotype of people with severe hemophilia under prophylaxis in order to better individualize the treatment.

REFERENCES

[1] Gouider E. Show me the evidence: Effectiveness of low-dose prophylaxis. Haemophilia 2019; 26:9-10.

[2] Gouider E, Jouini L, Achour M, et al. Low dose prophylaxis in Tunisian children with haemophilia. Haemophilia 2017; 23(1) :77-81.

[3] Pavlova A, Oldenburg J. Defining Severity of Hemophilia: More than factor levels. Semin Thromb Hemost 2013; 39(7):702-710.

[4] Oldenburg J. Optimal treatment strategies for hemophilia: achievements and limitations of current prophylactic regimens. Blood 2015; 125 (13):2038-2044.

[5] Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. J Thromb Haemost 2009; 7(3):413-420.

[6] Ahnström J, Berntorp E, Lindvall K, Björkman S. A 6-year follow-up of dosing, coagulation factor levels and bleedings in relation to joint status in the prophylactic treatment of haemophilia. Haemophilia 2004; 10(6): 689-697.

[7] Driessler F, Miguelino MG, Pierce GF, Peters RT, Sommer JM. Evaluation of recombinant factor VIII Fc(Eloctate) activity by thromboelastometry in a multicenter phase 3 clinical trial and correlation with bleeding phenotype.Blood Coagul Fibrinolysis 2017; 28(7) :540-550.

[8] Furukawa S, Nogami K, Ogiwara K, Yada K, Minami H, Shima M. Systematic monitoring of hemostatic management in hemophilia A patients with inhibitor in the perioperative period using rotational thromboelastometry. J Thromb Haemos 2014; 13(7) :1279-1284.

[9] Shima M, Matsumoto T, Ogiwara k.New assays for monitoring haemophilia treatment. Haemophilia 2008; 14 Suppl 3:83-92.

[10] CavigliaH, Narayan P, Forsyth A. Musculoskeletal problems in persons with inhibitors: how do we treat? Hemophilia 2012; 18 Suppl 4:54□60.

[11] Chitlur M, Wariier I, Rajpurka M et al .Thromboelatography in children with coagulation factor deficiencies. Br J Haematol 2008; 142(2):250-256

[12] B Sorensen,J Ingerslev. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. J Thromb Haemos 2004; 2(1):102-110.

[13] Nair SC, Dargaud Y , Chitlur M, Sruvastava A. Tests of global haemostasis and their applications in bleeding disorders. Haemophilia 2010; 16(5):85-92.

[14] Zetterberg E , Brolin K, Lindahl R, Knobe K, Berntorp E. Evaluation of prophylactic therapy in haemophilia with global coagulation tests. Haemophilia 2018; 24(1):10-13.

[15] Chitlur M, Rivard GE, Lillicrap D et al. Recommendations for performing thromboelastography/thromboelastometry in hemophilia: communication from the SSC of the ISTH. J Thromb Haemost 2014; 12(1):103-106.