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Letter to the Editors-in-Chief

The Thrombodynamics® analyzer: A new thrombin generation analyzer compared to the Calibrated Automated Thrombogram® in liver transplantation

Dear Editors-in-Chief,

The Thrombodynamics-4D® assay (TD4D) (HemaCore, Moscow, Russia) is an innovative videomicroscopy system allowing analysis of fibrin clot propagation and thrombin generation [1]. Its novelty mainly consists of the real-time spatial visualization of the clot formation departing from a localized zone where tissue factor (TF) is immobilized, thereby mimicking a damaged vessel in contact with a thin layer of non-stirred thermostatically-controlled (37 °C) plasma. The TD device has been completed by a second innovation, named the TD4D, which offers the parallel measurement of thrombin generation [1].

Any new method in clinical practice must answer two main questions. How does the new method correlate with reference methods? What is the added value of the new method? We tried to answer these questions by testing the TD4D in liver transplantation (LT), which is the treatment of irreversible acute or chronic liver failure. During liver failure, all the hemostatic steps are altered [2]. LT is thus well suited to evaluate the performance of the method, making it possible to compare the TD4D results with those from routine coagulation tests and thrombin generation assay by the Calibrated Automated Thrombogram® (CAT, Stago, Asnières, France). This study, carried out on frozen samples offering a wide range of alterations, is thus a preliminary proof-of-concept study evaluating the performance of the TD4D and its specific advantages for future potential use in different clinical settings.

The blood samples used for this observational study were taken from two series of normal volunteers who were withdrawn in our central phlebotomy unit by nurses only asking the patients for their absence of treatment and good healthy state (n = 23 for the TD4D method and n = 50 for all the other tests) and from LT patients (n = 150 from 30 consecutive adult patients, transplanted between January and June 2017). The study was approved by the CCP Sud-Ouest and Outremer III (n° DC 2016/142). LT patients and volunteers gave their informed oral consent. The study design, preanalytical steps and routine parameters have been described elsewhere [3]. Blood samples were performed at T0: after induction of general anesthesia and before surgical incision; T1: during dissection of the native liver, at the time of portal vena clamping; T2: during the anhepatic phase; T3: 30 min after graft revascularization; T4: at the beginning of skin closure. All blood samples were taken in BD Vacutainer® tubes containing sodium citrate 0.109 M (3.2%). While routine parameters were assessed by standard methods on fresh plasma (performed using an ACL TOP® 700 device (Werfen, Barcelona, Spain), using routine reagents from Werfen (Recombiplastin, aPTT SP, Fibrinogen QFA, Werfen Deficient Plasmas), more specialized methods were used in series on plasma stored aliquots. The pre-analytical phase consisted of a double centrifugation for 10 min, 2000 *g* and 2400 g, snap-freezing of the aliquots at −80 °C and thawing in a water bath at 37 °C for 5 min just before processing.

The TD4D assay was carried out according to the provider's instructions. For thrombin formation, the raw fluorescence signal, which is proportional to thrombin concentration, is averaged in the area 0.05–0.2 mm from the TF surface (60 pmol/m^2) and is transformed into a thrombin generation curve as a function of time described by the usual parameters: lag time (TD4D-Lag), time to maximum concentration (TD4D-Tmax), maximum thrombin concentration (TD4D-Cmax) and endogenous thrombin potential (TD4D-ETP).

The Calibrated Automated Thrombogram® (CAT) using the Thrombinoscope™ software from Thrombinoscope (BV, Maastricht, The Netherlands) and the PPP reagent (TF 5 pM and phospholipids 4 μM) was used according to the provider's instructions. It is currently the most commonly used method for the thrombin generation test [4]. It uses the same parameters to describe the thrombin generation curve as the TD4D, which are identified as CAT-Lag, CAT-Tmax, CAT-Cmax and CAT-ETP.

Quantitative data are expressed as median (IQR 25–75) or mean ± SD. Normality of distribution was assessed with the Shapiro-Wilk test. Normal distributed data were compared with the Student-*t*-test, and non-normal distributed data with the Mann-Whitney test. Evolution of biological parameters between the different sample times was studied with mix model ANOVA for repeated measures or with a Friedman test for paired data when appropriate. A $p < 0.05$ or adjusted with Bonferroni correction in the event of multiple comparison tests was considered statistically significant.

Multidimensional data management was performed with a hierarchical clustering analysis (HCA). Dendrograms were plotted to illustrate the distances between the laboratory parameters. Briefly, HCA is an iterative classification (or clustering) method whose results are usually represented as dendrograms. A dendrogram represents the distance from the root to a subtree. It indicates the dissimilarity (Ward's aggregation method) of the subtree on an axis from 0 to ∞: the shorter the height of the subtree, the more similar the parameters. This global overview of the relationships between parameters is further detailed by Pearson correlation studies. All statistical tests were performed with the XLSTAT 2017 package (Addinsoft, Paris, France).

LT patients were 21 men and 9 women, median age 61 [55–63] years. MELD score was 10 [8–19]. Indications for LT were hepatocellular carcinoma (HCC, n = 16), alcoholic cirrhosis (n = 6), non-alcoholic steato-hepatitis (NASH) (n = 3), post-hepatitis C cirrhosis (n = 2), miscellaneous (n = 3). Median blood loss was 1550 [1000 − 3000] ml. A total of 148 stored samples from our LT study were analyzed by TD4D.

Fig. 1a shows the evolution of CAT and TD4D thrombin curves from T0 to T4 calculated by pooling the data of all patients, sampling time by sampling time. Starting times (lag times) and Tmax were different between the CAT and TD4D thrombin methods owing to the stimulation design, but the quantitative parameters of the thrombin generation

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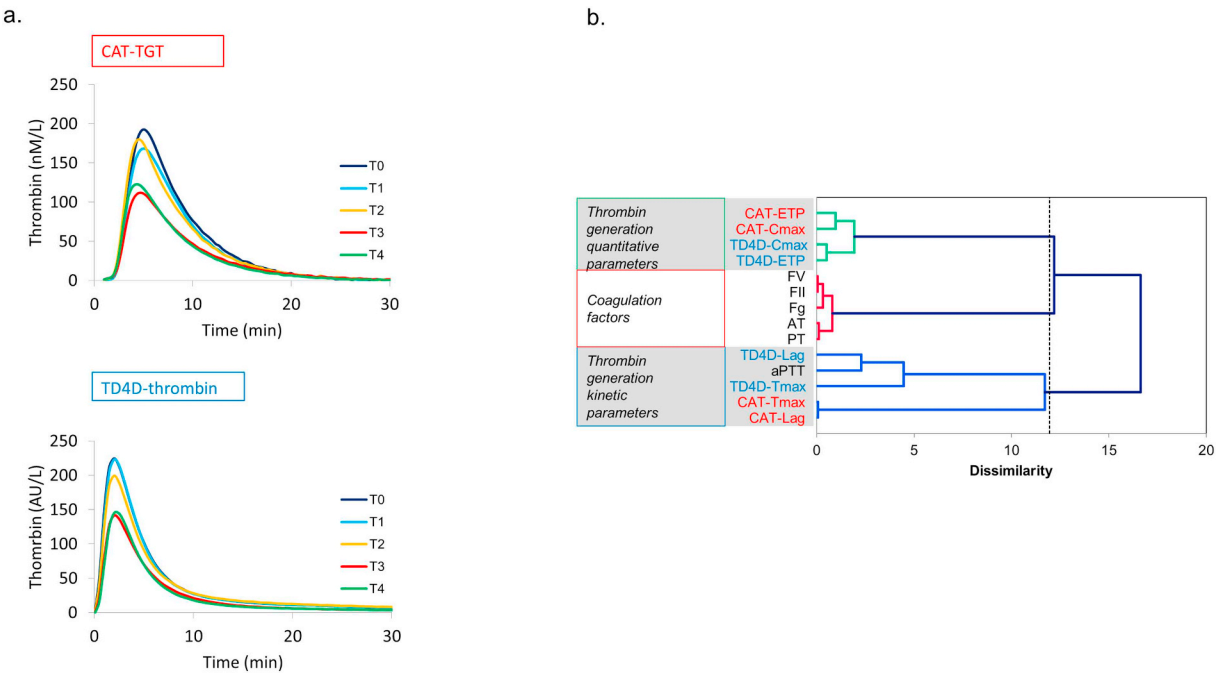

Fig. 1. Thrombin generation with CAT and TD4D
1a: Evolution from T0 to T4 of TD4D-thrombin and CAT-TGT curves calculated by pooling data of all patients, sampling time by sampling time.
1b: Dendrogram of hierarchical cluster analysis of biological parameters. aPTT: activated partial thromboplastin time, AT: antithrombin, Cmax: maximum concentration, CAT: Calibrated Automated Thrombogram, ETP: endogenous thrombin potential, FII: factor II, FV: factor V, Fg: fibrinogen, Lag: lag time, PT: prothrombin ratio, TD: Thrombodynamic fibrin clot parameters, TD4D: Thrombodynamic thrombin parameters, Tmax: time to maximum concentration.

curves (Cmax and ETP) were comparable with the two methods while they were very dissimilar among the sampling times.

Fig. 1b shows the results of the clustering of the different variables included in the study. It evidences three main sub-trees. The cophenetic correlation coefficient of the CAH was 0.822, reflecting the good reliability of the classification. A first sub-tree clusters the quantitative parameters thrombin generation (Cmax and ETP). All these factors are highly correlated ($p < 0.0001$) between quantitative parameters of thrombin generation (CAT-Cmax and TD4D-Cmax $R = 0.678$, CAT-ETP and TD4D-ETP $R = 0.524$, CAT-Cmax and CAT-ETP $R = 0.806$, TD4D-Cmax and TD4D-ETP $R = 0.890$), but also between all the above-mentioned TG parameters and the coagulation factors (PT, aPTT, Fibrinogen, factors II, V and antithrombin) which cluster in a second sub-tree. A last sub-tree clusters the kinetic parameters of thrombin generation as studied by CAT and TD4D (Lag time and Tmax) and aPTT. While they belong to the same cluster, the kinetic parameters were not significantly correlated between the two methods. On the contrary, the kinetic parameters (Lag time and Tmax) were highly correlated in the TD4D method ($R = 0.553$, $p < 0.0001$) and even more in the CAT method ($R = 0.961$, $p < 0.0001$).

Table 1 shows the results of CAT and TD4D in controls and patients at T0 and their evolution during LT. Alterations in thrombin generation were found in patients at T0 when compared to controls. During LT, all parameters were mostly preserved until T2, but at T3 major alterations occurred after graft revascularization.

In the setting of LT, TD4D allows individual evaluation of thrombin formation on plasma samples, and thrombin generation is in high accordance with the reference CAT method for thrombin formation. The lag time was highly influenced by the different stimulation design of the two methods, but the quantitative parameters of thrombin generation parameters were highly correlated. Stimulating coagulation from a localized area, as in the TD4D, mimics better the *in vitro* situation than mixing TF in a plasma volume (the homogeneous phase stimulation) [5]. Although localized stimulation induced very short TD4D lag times before the thrombin bursts, it was highly sensitive to the coagulation alterations exhibited at T3 and T4, where it was more significantly altered than the CAT lag time. Whatever the method, the different thrombin generation parameters underwent alterations similar to those described by Lisman et al. [6]: quite stable until the anhepatic phase and then a dramatic decrease after graft revascularization and at the end of surgery.

The results of our study answer our preliminary question: there is a coherent parallel between a highly referenced test that has been used for more than a decade (the CAT from Stago) and the new device, which is an interesting alternative for thrombin generation evaluation in individual samples.

The only published study in patients with severely altered liver function who used the TD to monitor clot growth found clot size to be unaltered, despite a significant decrease in clot density in Child C patients when compared to controls and Child A and B patients [7]. We confirmed in our study that clot size was not altered in our patients, while clot density was decreased and highly correlated with the fibrinogen (data not shown). A major drawback of the TD/TD4D method is the very high incidence of “spontaneous clots” which interfere with the clot density measurement [8] and requires a further data management of the raw data. It is important to underline that the spontaneous clots do not interfere with the thrombin generation analysis which takes place at the immediate vicinity of the TF-coated surface (0.05–0.2 mm). Indeed, raw data analysis showed that the shortest distance between these clots and the TF-coated surface was never shorter than 0.4 mm. The median value was 1.00 [IQR 25–75: 0.80–1.30] mm.

In conclusion, thrombin generation assessment is attracting increasing interest as a tool for recognizing coagulation alterations at all the phases of the coagulation process. CAT is not suitable for routine practice because of its analytical constraints. In our study, the TD4D mirrored the performance of the CAT. It is quite easy to utilize with an isolated sample and would be useful in emergency laboratories once the clinically relevant parameters are defined.

Table 1
Biological results of controls and patients at different sampling times during LT.

	Controls	Patients				
		T0	T1	T2	T3	T4
CAT-Thrombin generation test						
CAT_Lag (min)	2.7 (2.5–2.9)	2.7 (2.3–3.1)**	2.6 (2.3–3.2)	2.3 (2.1–2.7)*	2.3 (2.2–2.7)	2.2 (2.1–2.7)*
CAT_Cmax (nM)	303 (277–341)	211 (185–256)****	211 (175–246)	227 (178–247)	122 (105–154)*** §§§ $$$	142 (111–167)*** §§§ $$$
CAT_ETP (nM/min)	1448 (1292–1635)	1351 (1107–1517)*	1257 (1155–1411)	1300 (1069–1400)	862 (689–1058)*** §§§ $$$	861 (655–10,100)*** §§§ $$$
CAT_Tmax (min)	4.9 (4.7–5.2)	5.0 (4.4–5.6)	5.0 (4.3–5.9)	4.6 (4.2–5.1)	4.7 (4.3–5.3)	4.3 (4.0–4.7)**
TD4D -Thrombodynamics®						
Thrombin generation						
TD4D_Lag (min)	0.1 (0.1–0.2)	0.2 (0.2–0.4)****	0.3 (0.2–0.6)	0.4 (0.2–0.5)	0.6 (0.3–0.7)**	0.6 (0.5–0.8)*** § $
TD4D_Cmax (au/l)	304 (271–319)	233 (171–292)***	241 (162–272)	210 (144–269)	153 (114–187)*** §§§	139 (114–190)*** §§§ $
TD4D_ETP (au * min/l)	1558 (1408–1643)	1383 (863–1913)	1483 (871–1735)	1318 (792–1687)	800 (574–1056)*** §§§ $$$	749 (583–1112)*** §§§ $$$
TD4D_Tmax (min)	2.1 (1.6–2.1)	2.1 (2.1–2.1)*	2.1 (2.1–2.6)	2.1 (2.1–2.3)	2.1 (2.1–2.6)	2.1 (2.1–2.6)
Routine parameters						
Hb (g/dl)	15.1 (13.4–16.7)	10.3 (9.1–12.2)****	10.4 (9.1–12.0)	10.2 (9.2–11.4)	9.4 (8.8–10.6)* §	10.5 (9.0–11.2)
Plts (G/l)	271 (150–393)	89 (63–125)****	93 (71–140)	89 (71–127)	96 (76–124)	101 (82–138)
PT %	109 (103 − 121)	66 (45–81)****	62 (41–78)	59 (38–73)*	39 (26–49)*** §§§ $$	40 (28–49)*** §§§ $
aPTT (s)	30.0 (28.1–32.6)	32.9 (29.4–37.6)*	32.9 (28.8–42.9)	34.9 (28.4–41.6)	59.5 (48.2–75.6)*** §§§ $$$	49.3 (40.0–59.7)** §§§ $$$
Fg (g/l)	2.7 (2.4–3.0)	2.2 (1.8–3.0)*	2.0 (1.5–2.5)	1.9 (1.4–2.3)	1.2 (0.9–1.7)*** §§§ $$	1.4 (1.0–1.8)*** §§§
Factor II (%)	98 (91–107)	58 (43–70)****	54 (31–63)	47 (34–56)***	29 (19–37)*** §§§ $$$	26 (21–34)*** §§§ $$$
Factor V (%)	97 (91–108)	65 (46–87)***	61 (43–78)	55 (38–72)	27 (17–40)*** §§§ $	26 (13–38)*** §§§ $$$
Antithrombin (%)	104 (101 − 110)	53 (33–69)****	51 (27–67)	44 (24–57)***	28 (19–37)*** §§§ $$$	28 (21–37)*** §§§ $$$

Data are presented as median (IQR 25–75). au, arbitrary unit; Cmax, maximal concentration; ETP, endogenous thrombin potential; Hb, haemoglobin; Fg, fibrinogen; Plts, platelets; PT, prothrombin ratio; Tmax, time to maximal concentration.
For comparisons between controls and patients at T0, a p value < 0.05 is considered significant. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 *versus* controls.
For comparisons between T0, T1, T2, T3 and T4, according to Bonferroni's correction for multiple pairwise comparison, a p value < 0.005 is considered significant. *p < 0.005, **p < 0.001, ***p < 0.0001 *versus* T0; §p < 0.005, §§p < 0.001, §§§p < 0.0001 *versus* T1; $p < 0.005, $$p < 0.001, $$$p < 0.0001 *versus* T2; †p < 0.005, ††p < 0.001, †††p < 0.0001 *versus* T3.

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Declaration of interest

None.

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S. Roullet[a,b,*], S. Labrouche[c,d], L. Chiche[e], K. Nouette-Gaulain[a,b], C. Laurent[e], G. Freyburger[c]
[a] *CHU Bordeaux, Service d'Anesthésie-Réanimation 1, F-33000 Bordeaux, France*
[b] *Université de Bordeaux, INSERM U 12-11, Maladies rares: Génétique et Métabolisme, F-33000 Bordeaux, France*
[c] *CHU Bordeaux, Laboratoire d'hématologie – PTRR, Hôpital Pellegrin, F-33000 Bordeaux, France*
[d] *Université de Bordeaux, INSERM U 10-34, Biologie des Maladies Cardio-Vasculaires, F-33600 Pessac, France*
[e] *CHU Bordeaux, Service de chirurgie hépato-biliaire et transplantation hépatique, F-33600 Pessac, France*
E-mail address: stephanie.roullet@chu-bordeaux.fr (S. Roullet).

[*] Corresponding author at: Service Anesthésie Réanimation Pellegrin - Centre Hospitalier Universitaire de Bordeaux, Place Amélie Raba Léon, 33076 Bordeaux Cedex, France.