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## Assessment of coagulation utilizing thromboelastometry in dogs undergoing orthopedic surgery

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1 Assessment of coagulation in dogs undergoing orthopedic surgery utilizing thromboelastometry

2

### 3 **Abstract**

4 **Objective** – Evaluation of blood coagulation by means of thromboelastometry in dogs after  
5 orthopedic surgery.

6 **Design** – Longitudinal observational study.

7 **Setting** – University Veterinary Teaching Hospital.

8 **Animals** – Thirty-four adult, client-owned dogs.

9 **Interventions** – Whole blood from each dog was collected by jugular venipuncture (20-gauge  
10 needle) using minimum stasis. The blood was then placed into tubes containing 3.8% trisodium  
11 citrate (1 part citrate: 9 parts blood) and stored at 37°C.

12 **Measurements and Main Results** – Dogs undergoing orthopedic surgery were enrolled and  
13 whole blood was collected before (T0), at 24 hours (T1) and 1 week (T2) after surgery.

14 Statistically significant differences ( $p < 0.05$ ) between the values of the thromboelastometry  
15 parameters were noted: an increase in maximum clot firmness (MCF) from T0 to T1 in the in-  
16 TEM and fib-TEM profiles (both  $p=0.0001$ ), and from T0 to T2 in the in-TEM, ex-TEM, and  
17 fib-TEM profiles ( $p=0.012$ ,  $p=0.037$  and  $p=0.0001$ , respectively), and in the  $\alpha$  angle in the in-  
18 TEM and ex-TEM profiles ( $p=0.019$  and  $p=0.036$ , respectively), and in the fib-TEM profile from  
19 T1 to T2 ( $p=0.039$ ). All parameters were, however, within our institutional reference ranges.

20 **Conclusions** – This is the first study to assess changes in coagulability by means of  
21 thromboelastometry and platelet function analysis in dogs following orthopedic surgery. Our  
22 results show that, unlike the increased hypercoagulation observed in human orthopedic patients,  
23 a hypercoagulable state did not develop in dogs undergoing orthopedic surgery.

24

25 Key words: small animal, hemostasis, surgery, thromboelastometry.

26

27 aPTT activated partial thromboplastin time

28 CFT clot formation time

29 CT clotting time

30 MCF maximum clot firmness

31 PT prothrombin time

32 TEG thromboelastography

33 TEM thromboelastometry

34 THR total hip replacement

35

## 36 **Introduction**

37 Hypercoagulable states are frequent in human patients undergoing surgery. According to a study  
38 by McCrath *et al.* (2005), hypercoagulability following non cardiac surgeries develops in 40% of  
39 patients.<sup>1</sup> Such conditions, associated with other factors of Virchow's triad (i.e., venous stasis  
40 and vessel wall damage), may lead to thrombotic complications, including myocardial infarction,  
41 ischemic stroke, deep vein thrombosis and pulmonary embolism.<sup>1</sup>

42 Numbering among the categories of surgical patients considered at risk for thrombotic  
43 complications are those undergoing major orthopedic surgery (new and revision total hip  
44 replacement, total knee replacement or fractured neck of femur repair).<sup>2</sup> Studies conducted in  
45 human medicine have shown a prothrombotic state in surgical patients; for example, Wilson *et*  
46 *al.* (2001) evaluated hemostasis in 250 patients undergoing surgery for proximal femoral fracture  
47 and found hypercoagulability to be correlated with the development of deep venous thrombosis;

48 Okamura *et al.* (2008) observed hypercoagulability in 30 human patients undergoing total knee,  
49 total hip arthroplasty, and other lower extremity orthopedic surgeries.<sup>3,4</sup> The hypothesized causes  
50 for the hypercoagulability were surgical trauma with tissue factor expression, systemic  
51 inflammation, platelet activation, blood loss, and fluid administration.<sup>1,3</sup> Because of the risk of  
52 thrombosis, all human patients receive antithrombotic prophylaxis after orthopedic surgery.  
53 Hypercoagulability in dogs after orthopedic surgery has not yet been investigated. In veterinary  
54 medicine, a few studies in dogs have described pulmonary embolic complications following  
55 cemented total hip replacement (THR).<sup>5,6,7</sup> The pathogenic hypothesis for this event is the  
56 elevated femoral intramedullary pressure during stem insertion, ensuing in fat or bone marrow  
57 embolization.<sup>8</sup> Pulmonary embolism was not reported in a study on non cemented THR in 11  
58 dogs, where other surgical techniques were applied and pulmonary embolism was diagnosed  
59 differently.<sup>9</sup>

60 Hypercoagulability in postsurgical human patients has been investigated by  
61 thromboelastography. Thromboelastography (TEG)/thromboelastometry (TEM) measure the  
62 viscoelastic properties of whole blood during the various different phases of clot formation,  
63 stabilization and eventual lysis. This complete view of the entire hemostatic process makes the  
64 techniques a good instrument to study hypercoagulability. In veterinary medicine,  
65 hypercoagulability has been investigated and demonstrated by means of TEG in a variety of  
66 disorders, including parvoviral infection, neoplasia, protein-losing enteropathy, hemolytic  
67 anemia, disseminated intravascular coagulation and protein-losing nephropathy.<sup>10-15</sup> Recently,  
68 Smith *et al.* validated TEM also for the canine species.<sup>16</sup>

69 Knowing the hemostatic status and its related potential complications is important, especially in  
70 intensive care unit patients. In brief, TEM/TEG are new tools for the complete assessment of  
71 coagulation.

72 The aim of this study was the perioperative evaluation of blood coagulation by means of TEM in  
73 dogs undergoing orthopedic surgery. Our hypothesis was that in dogs, as in humans, orthopedic  
74 surgery may cause hypercoagulability.

75

## 76 **Materials and methods**

### 77 **Animals**

78 The study was conducted according to animal welfare considerations and regulations of the  
79 Ministry of Health. Dogs undergoing orthopedic surgery between January and September 2009  
80 were enrolled into this prospective clinical study after informed consent was obtained from the  
81 owners. The dogs underwent THR, THR revision, double pelvic osteotomy, tibial plateau  
82 leveling osteotomy, femoral fracture repair or elbow fracture repair.

83 The exclusion criteria were: presence of neoplasia, history of a tendency to spontaneous  
84 bleeding; positivity to serologic tests for *Leishmania infantum* (titer >1:40; immunofluorescence  
85 antibody test), for *Ehrlichia canis*<sup>a</sup>, *Borrelia burgdorferi*<sup>a</sup>, *Anaplasma phagocytophilum*<sup>a</sup> or  
86 *Dirofilaria immitis*<sup>a</sup>; administration of corticosteroids in the 4 weeks before surgery.

87 The patients underwent preoperative evaluation including: physical examination; complete blood  
88 count<sup>b</sup>; biochemical profile<sup>c</sup> including albumin, total protein, blood urea nitrogen, creatinine,  
89 glucose, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase,  $\gamma$ -glutamyl  
90 transpeptidase, cholesterol, triglyceride and urinalysis (dipstick test<sup>d</sup> and sediment analysis).

91 Dogs were premedicated with eptadone (0,2 mg/Kg IM) and anesthesia was induced with  
92 propofol (2 to 4 mg/Kg IV, to effect). Dogs then were intubated, and anesthesia was maintained  
93 by administration of isoflurane in oxygen and air. All dogs were administrated lactated Ringer's  
94 solution at a rate of 10 ml/ Kg/h IV. All surgeries were performed by one experienced surgeon  
95 (LP) and standardized surgical protocols were used. After the extubation standard postoperative  
96 care included the administration of buprenorfine (10 µg/Kg/6-hourly, IV) and carprofen (2  
97 mg/kg/12-hourly, SC or orally with food).

98 The sample size had been determined using the Kastenbaum, Hoel e Bowman tables for  
99 ANOVA;<sup>17</sup> A minimum sample size of 25 animals, repeated for three measurements, was  
100 calculated, taking into account: a) a power of the study equal to 80%; b) a significance level of  
101 0.05; c) a standardised range (max-min/sigma) equal to 0.8.

102

### 103 **Hemostasis**

104 Thromboelastometry,<sup>e</sup> PFA-100<sup>f</sup> and platelet count (CBC)<sup>b</sup> were performed at three time points:  
105 1 hour before the surgery (T0), 24 hours after the conclusion of the surgery (T1), and 7 days after  
106 surgery (T2).

107 Blood specimens were collected by jugular venipuncture with a 20 gauge needle by exerting  
108 minimal hemostasis on the vessel. Samples obtained with difficulty (e.g. venipuncture requiring  
109 numerous attempts, repositions of the needle or interruption in blood flow into the tube) were  
110 discarded and the collection was repeated in the contralateral jugular. Samples were stored at  
111 37°C in 3.8% trisodium citrate tubes.<sup>g</sup>

112 For the thromboelastometry assay, analyses were performed within 30 minutes after blood  
113 collection according to the manufacturer's instructions, and the analyses were run for 60

114 minutes. Three different profiles were tested for each sample: in-TEM, ex-TEM and fib-TEM  
115 assays. In the in-TEM assay, the sample is recalcified by the star-TEM<sup>h</sup> reagent and the intrinsic  
116 pathway is activated by the in-TEM reagent<sup>i</sup>, whereas in the ex-TEM profile, after  
117 recalcification, the extrinsic pathway is triggered by the ex-TEM reagent<sup>l</sup>. In the fib-TEM assay,  
118 the extrinsic pathway is activated by tissue factor in the presence of a platelet inhibitor<sup>m</sup> to assess  
119 the functional fibrinogen level. The following parameters were assessed for each profile: clotting  
120 time ([CT], s); clot formation time ([CFT], s); maximum clot firmness ([MCF]; mm) and  $\alpha$  angle  
121 ( $\alpha$ , °).

## 122 **Statistical analysis**

123 The data were entered into an *ad hoc* database and analyzed using **commercial statistical**  
124 **software<sup>h</sup>**. A test for normality based on skewness and on kurtosis was performed to test data  
125 distribution. Levene's robust test was used to evaluate the homogeneity of variances. ANOVA  
126 was applied to the data to compare the lengths of coagulation time. **The Bonferroni's correction**  
127 **was applied**. When the data did not fulfill the assumptions of the parametric method, Friedman's  
128 two way analysis of variance was performed. The significance level was set at  $p < 0.05$ .

129

## 130 **Results**

131 Of 34 eligible adult dogs candidates for orthopedic surgery, 29 were included at T0 and T1 and  
132 25 at T2 (4 animals were lost to follow-up because the owners did not return for the second  
133 visit), and 5 were excluded (1 because of neoplasia, 1 because of filariasis and 3 because of  
134 Leishmaniasis). **Seven dogs underwent THR, 1 THR revision, 1 double pelvic osteotomy, 16**  
135 **tibial plateau leveling osteotomy, 3 femoral fracture repair and 1 elbow fracture repair.**

136 Of these 29 dogs, 13 were males and 16 females, aged from 1 to 11 years (age,  $3.64 \pm 2.77$ ).  
137 Four dogs were crossbreed, 6 were Labrador Retriever, 2 were Beagle, 1 Cane Corso and 1  
138 German Shepherd; the other breeds included: Boxer, Bull Mastiff, English Bull Dog, Dobermann  
139 Pinscher, Dogue de Bordeaux, **Drahthaar (German wire-haired pointer)**, Golden Retriever,  
140 Maremma sheepdog, American Pit Bull Terrier, Setter Gordon, and Sharpei. The CBC,  
141 biochemical and urinalysis values were all within our institutional reference ranges.  
142 The results of the comparisons of the TEM tracings at the three time points (T0 vs T1, T1 vs T2  
143 and T0 vs T2) are listed in Tables 1-3, respectively. Significant differences ( $p < 0.05$ ) were found  
144 between T0 and T1, where there was an increase in MCF in the in-TEM and fib-TEM profiles at  
145 T1; between T0 and T2, where there was an increase in MCF (in all profiles) and the  $\alpha$  angle (in  
146 the in-TEM and ex-TEM profiles) at T2; between T1 and T2, where MCF was increased in the  
147 fib-TEM profile at T2. All parameters were within our institutional reference ranges, however  
148 (Table 4).<sup>18</sup>

149

## 150 **Discussion**

151 Orthopedic surgery is known to increase the risk for hypercoagulability and thromboembolic  
152 complications during the postsurgical period in human patients.<sup>3,4</sup>

153 To the best of the authors' knowledge, coagulation in perioperative dogs has been assessed in a  
154 few studies and with different methods. Two studies evaluated the blood coagulation profile after  
155 ovariohysterectomy in female dogs: Millis *et al.* (1992) performed standard coagulation profiles  
156 (PT, aPTT and fibrinogen), fibrin degradation product, antithrombin III and platelet count;  
157 Sobiech *et al.* (2011) carried out standard coagulation profiles, thrombin time, D-dimer and  
158 antithrombin activity.<sup>19,20</sup> The first study revealed only a postoperative increase in fibrinogen



159 level, whereas the second showed a prolonged aPTT, higher fibrinogen and D-dimer  
160 concentrations and lower levels of antithrombin activity in the postoperative patient.<sup>19,20</sup> Another  
161 study in dogs after gonadectomy evaluated the bleeding tendency in greyhounds according to  
162 platelet count, PFA-100, von Willebrand factor, factor VIII, PT, aPTT, fibrinogen, D-dimer,  
163 plasminogen, antiplasmin and antithrombin. The results showed a post-operative increase in the  
164 fibrinogen level and antiplasmin activity.<sup>21</sup> Altered fibrinolysis was reported by Lanevschi *et al.*  
165 (1996) who evaluated plasminogen, tissue plasminogen activator and alpha 2-antiplasmin in dogs  
166 after different surgical procedures. Finally, a recent study by Villar *et al.* (2011) showed that  
167 aPTT and PT are not predictors of bleeding in greyhounds undergoing gonadectomy, while  
168 thromboelastography parameters representing fibrin cross-linking ( $\alpha$  angle) and clot strength  
169 (maximum amplitude) were considered predictors of bleeding. Indeed, postsurgical TEG showed  
170 a decrease in the  $\alpha$  angle in the bleeder dogs and an increase in the maximum amplitude and  $\alpha$   
171 angle in the non-bleeder dogs.<sup>22</sup>

172 Thromboelastometry/thromboelastography are useful tools to identify hypo- and  
173 hypercoagulable conditions in dogs.<sup>10,11,14,23,24</sup> In the thromboelastometric profiles, CT represents  
174 the first phase of fibrin formation, from activation of the test to a clot amplitude of 2 mm; this  
175 parameter is mainly affected by the concentration of plasma coagulation factors and coagulation  
176 inhibitors (e.g., antithrombin or drugs).<sup>25,26</sup> CFT expresses the velocity of clot formation and is  
177 affected predominantly by platelet number and function and by fibrinogen activity. MCF, the  
178 maximum firmness reached by the clot, is determined by both platelet number and function and  
179 fibrin formation in the presence of factor XIII.<sup>25,26</sup> The  $\alpha$  angle corresponds to the slope of the  
180 tangent on the elasticity curve, where a decrease indicates a tendency towards hypocoagulability  
181 and an increase a hypercoagulable condition.<sup>25,26</sup>

182 The TEM profiles in our study showed changes indicating an increase towards a prothrombotic  
183 state in dogs undergoing orthopedic surgery; nonetheless, all parameters were within our  
184 institutional reference ranges.<sup>18</sup> These changes, as indicated by the increase in MCF and the  $\alpha$   
185 angle, are similar to those Villar *et al.* (2011) reported for the TEG profile after gonadectomy in  
186 non-bleeder greyhounds. Also in human studies, TEG showed a greater increase in maximum  
187 amplitude (the TEG parameter corresponding to MCF) and  $\alpha$  angle, indicating a condition of  
188 hypercoagulability. Wilson *et al.* (2001) identified, in patients following surgery for proximal  
189 femoral fracture, a period of hypercoagulability that persisted for 6 weeks, despite the use of  
190 antithrombotic prophylaxis. More recently, McCrath *et al.* (2005) reported that the incidence of  
191 thrombotic complications in patients undergoing a wide variety of surgical procedures was  
192 significantly more frequent, with a maximum amplitude >68 mm.<sup>1,3</sup>

193 MCF results from the interaction between platelets and fibrinogen activation in the presence of  
194 factor XIII, and it does not depend on the presence of procoagulant factors. An increase in this  
195 parameter can be due to an increase in fibrinogen concentration, in platelet activity or in the level  
196 or activity of factor XIII. Finally, alterations in TEG parameters (prolonged clot formation time  
197 and decreased  $\alpha$  angle) following carprofen administration, previously reported by Brainard *et*  
198 *al.*, were not identified in the present study.<sup>27</sup>

199 This is the first study to assess coagulation status by means of thromboelastometry in dogs  
200 following orthopedic surgery. Contrary to what happens in human orthopedic patients,  
201 hypercoagulability did not develop in our study population. In human medicine, the mechanisms  
202 thought to cause hypercoagulability are surgical trauma with tissue factor expression, systemic  
203 inflammation, platelet activation, blood loss and fluid administration.<sup>1,3</sup> Further studies are

204 needed to explain why a hypercoagulable state does not occur in dogs, despite the presence of at  
205 least some of such predisposing factors.

206 Our results could mean that healthy dogs after orthopedic surgery might be less predisposed than  
207 human patients to thrombus formation.<sup>28,29</sup> Venous studies with contrast (e.g., angiography or  
208 computed tomography angiography) might be one way to exclude the presence of  
209 thromboembolic events, obviating the need antithrombotic prophylaxis in orthopedic  
210 postoperative dogs admitted to an intensive care unit.

211 Finally, further studies are needed to compare the impact of different orthopedic surgeries, the  
212 changes in coagulability in a population of older dogs, and the interaction of concomitant  
213 pathologies or other predisposing factors (e.g., patients with multiple trauma).

214

#### 215 *Footnotes*

216 <sup>a</sup> Snap 4 DX, IDEXX Laboratories, Westbrook, ME, USA.

217 <sup>b</sup> ADVIA 120 Hematology, Siemens Healthcare Diagnostics, Tarrytown, NY, USA.

218 <sup>c</sup> ILAB 300 plus, Clinical Chemistry System, Instrumentation Laboratories, Milan, Italy.

219 <sup>d</sup> Multistix 10 SG Reagent Strips, Siemens Healthcare Diagnostics, Tarrytown, NY, USA.

220 <sup>e</sup> ROTEM, TEM innovation GmbH, Munich, Germany.

221 <sup>f</sup> Venosafe 3.8% buffered sodium citrated, Terumo, Leuven, Belgium.

222 <sup>g</sup> Stata Statistical Software: Release 11. StataCorp LP, College Station, TX, USA.

223 <sup>h</sup> Star-TEM 10 (0.2 mol/l CaCl<sub>2</sub> in HEPES buffer pH 7.4 and 0.1% sodium acide in glass  
224 vials), TEM innovations GmbH- Munich-Germany.

225 <sup>i</sup> In-TEM (partial thromboplastin phospholipid made of rabbit brain (chloroform extract),  
226 ellagic acid, buffer, preservatives in small glass vials), TEM innovations GmbH- Munich-  
227 Germany.

228 <sup>l</sup> Ex-TEM (recombinant tissue factor and phospholipids, CaCl<sub>2</sub>, preservatives and buffer in  
229 small glass vials), TEM innovations GmbH- Munich-Germany.

230 <sup>m</sup> Fib-TEM (Cytochalasin D / DMSO solution 0.2 mol/l CaCl<sub>2</sub> in HEPES buffer pH 7.4,  
231 preservative in glass vials), TEM innovations GmbH- Munich-Germany.

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327 **Table 1:** Comparison between thromboelastometry values obtained at T0 (n=29) and T1 (n=29).

	CT <sup>§</sup> s		CFT <sup>  </sup> s		MCF <sup>¶</sup> mm		$\alpha$ <sup>**</sup> degree	
	T0	T1	T0	T1	T0	T1	T0	T1
<b>in-TEM</b>	178.8 (118-390)	176.03 (134-263)	96.83 (52-202)	78.67 (47-145)	63.23 (53-74)	68.8* (59-86) p=0.0001	72.27 (55-80)	75.5 (62-81)
<b>ex-TEM</b>	49.59 (31-81)	48.59 (32-66)	111.34 (60-215)	95.59 (53-223)	61.48 (48-75)	67.86 (49-78)	69.03 (55-79)	71.48 (51-80)
<b>fib-TEM</b>	48.7 (25-77)	48.26 (26-84)	na	na	15.76 (5-36)	18.4* (6-35) p=0.0001	68.29 (50-82)	73.85 (63-81)

328



329 Values are expressed as median (minimum-maximum); na, not applicable.

330 \* statistically significant differences between the control and the postsurgical group ( $p < 0.05$ );

331 §clotting time; || clot formation time; ¶ maximum clot firmness; \*\* $\alpha$  angle.

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337 **Table 2:** Comparison between thromboelastometry values obtained at T1 (n=29) and T2 (n=25).

	CT <sup>§</sup> s		CFT <sup>  </sup> s		MCF <sup>¶</sup> mm		$\alpha$ <sup>**</sup> degree	
	T1	T2	T1	T2	T1	T2	T1	T2
<b>in-TEM</b>	176.03 (134-263)	160.54 (118-224)	78.67 (47-145)	62.38 (37-92)	68.8 (59-86)	69.07 (56-79)	75.5 (62-81)	78.38 (73-82)
<b>ex-TEM</b>	48.59 (32-66)	43.84 (33-55)	95.59 (53-223)	70.56 (41-129)	67.86 (49-78)	69.6 (55-78)	71.48 (51-80)	76.04 (65-82)
<b>fib-TEM</b>	48.26 (26-84)	41.57 (33-55)	na	na	18.4 (6-35)	25.96* (13-36) p=0.039	73.85 (63-81)	75.34 (61-83)

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339 Values are expressed as median (minimum-maximum); na, not applicable.

340 \* statistically significant differences between the postsurgical groups ( $p < 0.05$ );

341 § clotting time; || clot formation time; ¶ maximum clot firmness; \*\* $\alpha$  angle.

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347 **Table 3:** Comparison between thromboelastometry values obtained at T0 (n=29) and T2 (n=25).

	CT <sup>§</sup> s		CFT <sup>  </sup> s		MCF <sup>¶</sup> mm		$\alpha$ <sup>**</sup> degree	
	T0	T2	T0	T2	T0	T2	T0	T2
<b>in-TEM</b>	178.83 (118-390)	160.54 (118-224)	96.83 (52-202)	62.38 (37-92)	63.23 (53-74)	69.08* (56-79) p=0.012	72.26 (55-80)	78.38* (73-82) p=0.019
<b>ex-TEM</b>	49.59 (31-81)	43.84 (33-55)	111.34 (60-215)	70.56 (41-129)	61.48 (48-75)	69.6* (55-78) p=0.037	69.03 (55-79)	76.04* (65-82) p=0.036
<b>fib-TEM</b>	48.7 (25-77)	41.58 (33-55)	na	na	15.76 (5-36)	25.96* (13-36) p=0.0001	68.29 (50-82)	75.34 (61-83)

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349 Values are expressed as median (minimum-maximum); na, not applicable.

350 \* statistically significant differences between the control and the postsurgical group ( $p < 0.05$ );

351 § clotting time; || clot formation time; ¶ maximum clot firmness; \*\* $\alpha$  angle.

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356 **Table 4:** Comparison of our institutional reference ranges for ROTEM tests (n=45) and values  
 357 measured at T0, T1 and T2.

Test	in-TEM				ex-TEM	
	T0	T1	T2	Range	T0	T1
CT <sup>s</sup> <sub>§</sub>	178.83	176.03	160.54	126-363	49.59	48.59
CFT <sup>s</sup> <sub>  </sub>	96.83	78.67	62.38	47-224	111.34	95.59
MC <sup>F</sup> <sub>¶</sub> mm	63.23	68.8	69.08	50-75	61.48	67.86
$\alpha$ <sup>°</sup> <sub>**</sub>	72.26	75.5	78.38	55-81	15.76	71.48

		<b>Fib-TEM</b>				
		T0	T1	T2	Range	
T2	Range					
43.84	29-92	48.7	48.26	41.58	14-102	
70.56	54-275	na*	na*	na*	na*	
69.6	36-73	15.76	18.4	25.96	6-26	
76.04	47-79	68.29	73.85	75.34	48-78	

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359 T0, T1 and T2 values are expressed as median; Range values are expressed as 5<sup>th</sup>-95<sup>th</sup> percentile  
 360 (95% confidence intervals); \* not applicable;

361 § clotting time; || clot formation time; ¶ maximum clot firmness; \*\* $\alpha$  angle.

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