

Hypothermia and Acidosis Synergistically Impair Coagulation in Human Whole Blood

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BACKGROUND: Hypothermia and acidosis were reported to influence coagulopathy in different clinical settings. We evaluated whole blood coagulation to determine the effects of hypothermia and/or acidosis on hemostasis.

METHODS: Whole blood samples (3.000 μ L) from 10 healthy volunteers (2 female, 8 male) were acidified by adding 40 μ L of hydrochloric acid of increasing molarity to achieve a blood pH (α -stat) between 7.0 and 7.37, and coagulation was analyzed by rotational thromboelastometry after an incubation period of 30 min using both intrinsically (InTEM™) and extrinsically (ExTEM™) activated assays. To assess temperature-dependent effects, all tests were performed at blood/thromboelastometer temperatures of 30, 33, 36, and 39°C, respectively. An additional extrinsically activated test with addition of cytochalasin D was performed to examine clot formation without platelet contribution.

RESULTS: Hypothermia at a normal pH produced an increased coagulation time [ExTEM: 65 s \pm 3.6 (36°C) vs 85 \pm 4 (30°C), $P < 0.001$; coagulation time, InTEM: 181 s \pm 10 (36°C) vs 226 \pm 9, $P < 0.001$] and clot formation time [ExTEM: 105 s \pm 5 (36°C) vs 187 \pm 6 (30°C), $P < 0.001$]; clot formation time [InTEM: 101 s \pm 5 (36°C) vs 175 \pm 7, $P < 0.001$], as well as decreased α angle [ExTEM: 65.6 \pm 1.8 (36°C) vs 58 \pm 1.1, $P < 0.01$, $P < 0.01$; InTEM: 70.5 \pm 1.8 (36°C) vs 60.2 \pm 1.5, $P < 0.001$]. Maximum clot firmness was significantly impaired only in InTEM assays [56.9 mm \pm 0.9 (36°C) vs 52.7 \pm 0.9, $P < 0.05$]. In contrast, acidosis *per se* had no significant effects during normothermia. Acidosis amplified the effects of hypothermia, and synergistically impaired clotting times, α angle, and decreased maximum clot firmness, again in both extrinsically and intrinsically activated assays. Formation of a fibrin clot tested after abolition of platelet function by cytochalasin D was not impaired. Clot lysis decreased under hypothermic and/or acidotic conditions, but increased with hyperthermia.

CONCLUSIONS: In this *in vitro* study, hypothermia produced coagulation changes that were worsened by acidosis whereas acidosis without hypothermia has no significant effect on coagulation, as studied by thromboelastometry. This effect was mediated by the inhibition of coagulation factors and platelet function. Thus, thromboelastometry performed at 37°C overestimated integrity of coagulation during hypothermia in particular in combination with acidosis.

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Massive hemorrhage in patients with major trauma is the second most common cause of death in the prehospital setting¹ and the most common cause of in-hospital mortality during the first 48 h² and in the early postoperative period.³ The highest mortality occurs in patients with hypothermia, acidosis, and coagulopathy.⁴⁻⁹ This combination is commonly referred to as the "lethal triad of trauma."^{4,5,10}

Clotting times of plasma, such as the activated partial thromboplastin time, prothrombin time (PT),

and thrombin time, are prolonged when assays are performed at lower temperatures in hypothermic patients, experimental animals, or plasma cooled *in vitro*.¹¹⁻¹⁶ Although these standard clotting tests are performed at 37°C, it has been suggested that they should rather be performed at the patient's core temperature.^{11,12,15,17,18} In contrast, Wolberg et al. reported that coagulation enzyme activities, as well as platelet activation, are not significantly decreased at 33°C versus 37°C.¹⁹

The role of acidosis in the development of clinical coagulopathy is even less investigated and studies have reported inconsistent results.^{4,20} Decreased clotting times have been reported in dogs after infusion of lactic acid,^{21,22} whereas Dunn et al. reported an increase in PT and PTT and a decrease in fibrinogen concentration and platelet count after hydrochloric acid infusion.²³

We reasoned that impairment of coagulation should be assessed in whole blood, as studied by rotational

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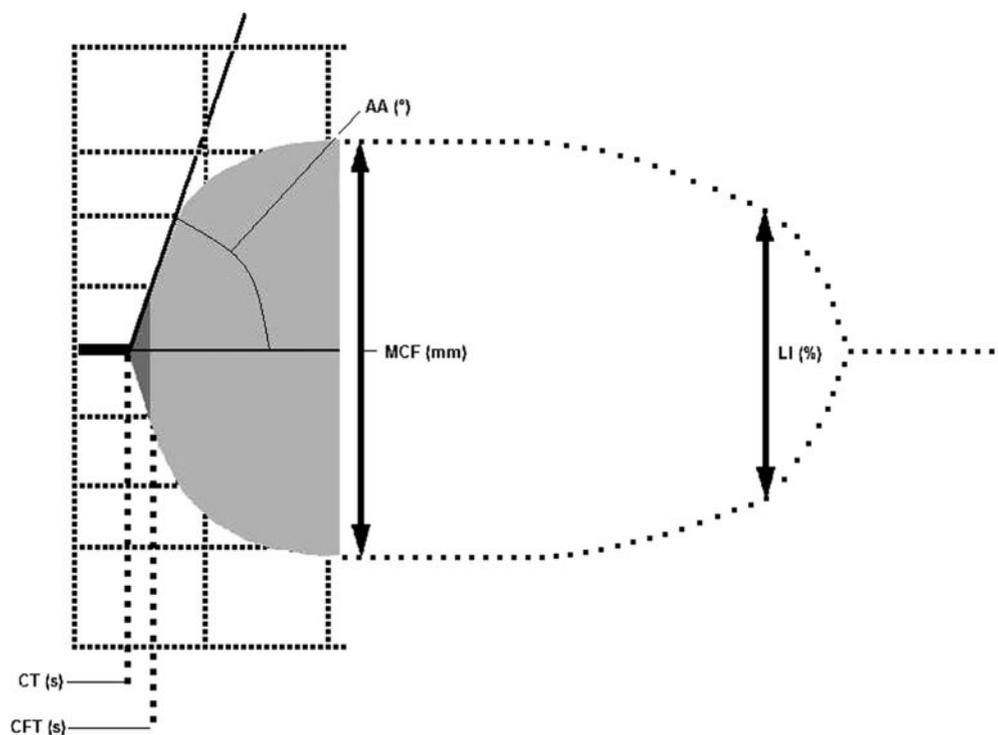


Figure 1. Typical rotational thromboelastometry (ROTEMTM) tracing, explaining the measured variables. Coagulation time (CT) (s) indicates the time from the start of the reaction until the clot has reached a 2 mm strength; clot formation time (CFT) (s) indicates the time from the end of CT until clot firmness has reached an amplitude of 20 mm; AA is the angle measured between the horizontal midline and a tangential to the graph; MCF (mm) is the maximum clot strength reached during measurements; LI (%) indicates the amount of the MCF still present after a certain time, for example after 60 min.

thromboelastometry (ROTEMTM), rather than by isolated assays and that it would be worthwhile to study the effects of hypothermia and acidosis, alone and combined. Specifically, we tested the hypotheses that both hypothermia and acidosis contribute to coagulopathy, and that their combination has synergistic effects.

METHODS

After ethics committee approval and informed consent, blood was drawn from 10 healthy volunteers (2 female, 8 male, age: 34.4 yr \pm 8.8) into sodium citrate-containing tubes using a 12-gauge IV catheter, thereby avoiding venous stasis. All volunteers had a negative history for a bleeding or prothrombotic diathesis, and denied the intake of anticoagulants or antiplatelet medication. All individuals showed normal values for the standard coagulation tests (PT, PTT, plasma fibrinogen concentration, platelet count, hemoglobin concentration, and hematocrit).

Measurements

To assess the effects of hypothermia, tubes were incubated for 30 min at specified temperatures of 30, 33, 36, and 39°C, respectively, and subsequent analyses were performed at these thromboelastometer temperatures. To assess the effects of acidosis, we slowly added to the tubes containing 3.000 μ L blood either 40 μ L of saline or hydrochloric acid of increasing molarity (0, 25, 0, 5, and 1 mol/L, respectively) to achieve a blood pH (measured at 37°C) of 7.37, 7.2, 7.1, and 7.0,

respectively. To assess combined effects both methods were used.

Coagulation was analyzed by ROTEM (Pentapharm GmbH, Munich, Germany) which is based on the original thrombelastography system (TEGTM) described by Hartert.²⁴ Technical details of ROTEM are described elsewhere,^{25,26} and the measurements were performed according to the manufacturer's instructions. Blood samples were recalcified using 20 μ L of CaCl₂ 0.2 M (Star-TEMTM) and assays were activated using either 20 μ L of tissue thromboplastin (i.e., phospholipids and tissue factor, ExTEMTM) or 20 μ L of partial thromboplastin (i.e., egalicacid and phospholipids, InTEMTM). In addition, cytochalasin D was added to another extrinsically activated assay to assess the plasmatic component of the clot strength, i.e., fibrin gelation and polymerization, without platelet contribution (FibTEMTM).²⁷ All ROTEM reagents were purchased from Pentapharm GmbH. ROTEM assays were performed for 60 min and data stored in a PC.

The following variables were determined: coagulation time (CT) corresponding to the reaction time (r time) of conventional TEG, clot formation time (CFT) corresponding to the CT (k time), α -angle (AA), maximum clot firmness (MCF) corresponding to the maximum amplitude, and the lysis index 60 (LI60 [%]), indicating the degree of the MCF still present after 60 min (Fig. 1).

The pH of each assay was determined by an electrode at 37°C (α -stat) (ABL FLEX-800 blood gas analyzer, Radiometer, Copenhagen, Denmark).

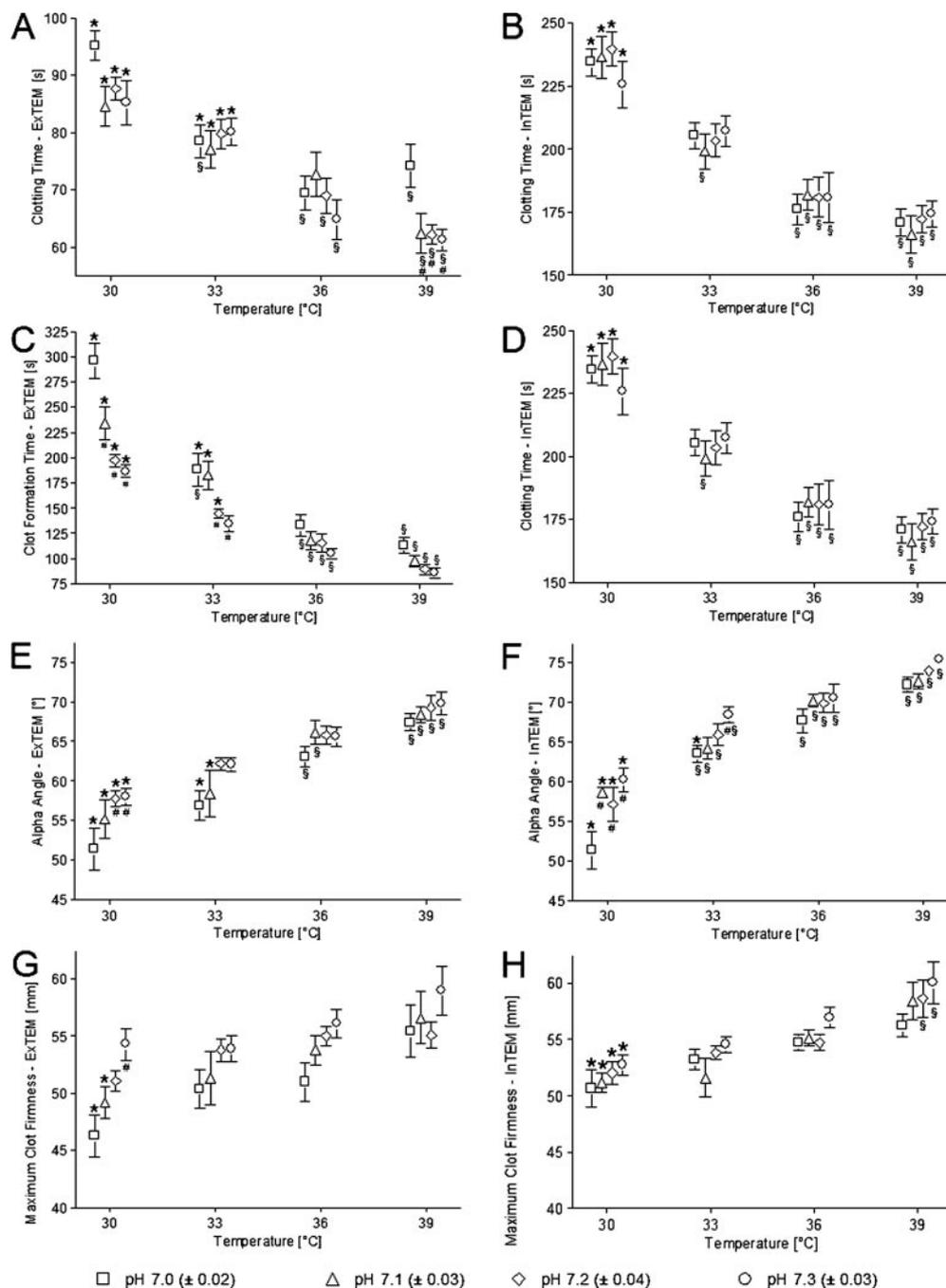


Figure 2. Effects on coagulation variables of hypothermia and acidosis, and of hypothermia/acidosis combined. Coagulation time (CT) in ExTEM™ (A), CT in InTEM™ (B), clot formation time (CFT) in ExTEM (C), CFT in InTEM (D), α angle (AA) in ExTEM (E), AA in InTEM (F), MCF in ExTEM (G) and maximum clot firmness (MCF) in ExTEM (H) in the different settings. *statistically significant compared with the control value at 36°C and pH 7.36 (± 0.03); #statistically significant to pH 7.0 (± 0.02) at same temperature; §statistically significant to 30°C at same pH.

Statistical Analysis

All data are shown as means (\pm standard error of the mean, SEM). A repeated measures analysis of variance (ANOVA) with Bonferroni-Holm adjustment for multiple tests was applied to assess the influence of different combinations of hypothermia and acidosis compared to the values at 36°C and pH 7.36 ± 0.03 . An α error P of $<0.05/n$ was considered statistically significant. In addition, we tested for combined effects of pH and temperature using a two-way ANOVA.

RESULTS

Hypothermia impaired coagulation in whole blood. In extrinsically and intrinsically activated tests, CT (Figs. 2A and B) and CFT (Figs. 2C and D) progressively increased and the AA (Figs. 2E and F) and MCF (Figs. 3A and B) progressively decreased with increasing hypothermia at a normal pH (7.36 ± 0.03). Compared with control values (36°C, pH 7.36 ± 0.03), all variables (except for MCF in ExTEM assays) were significantly impaired at 30°C. CT in ExTEM assay

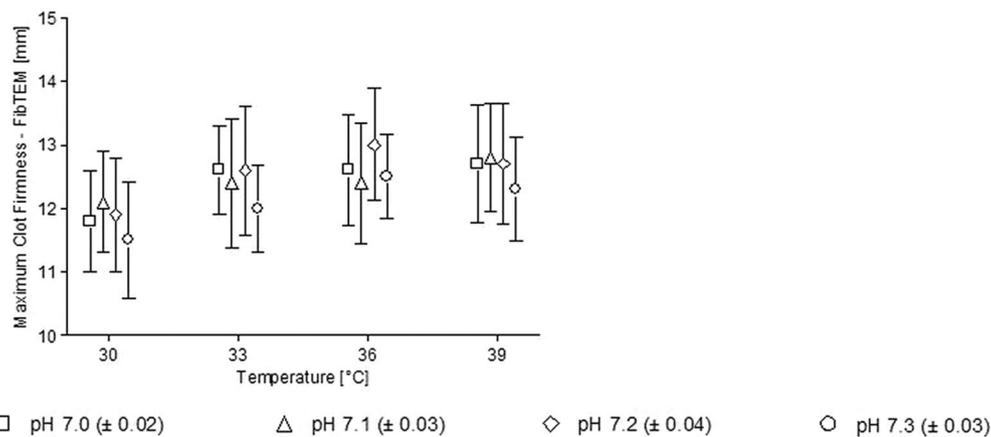


Figure 3. Effects on maximum clot firmness (MCF) of hypothermia and acidosis, and of hypothermia/acidosis combined studied with extrinsic activation and addition of cytochalasin D (FibTEM™) in the different groups. There were no significant differences between test conditions.

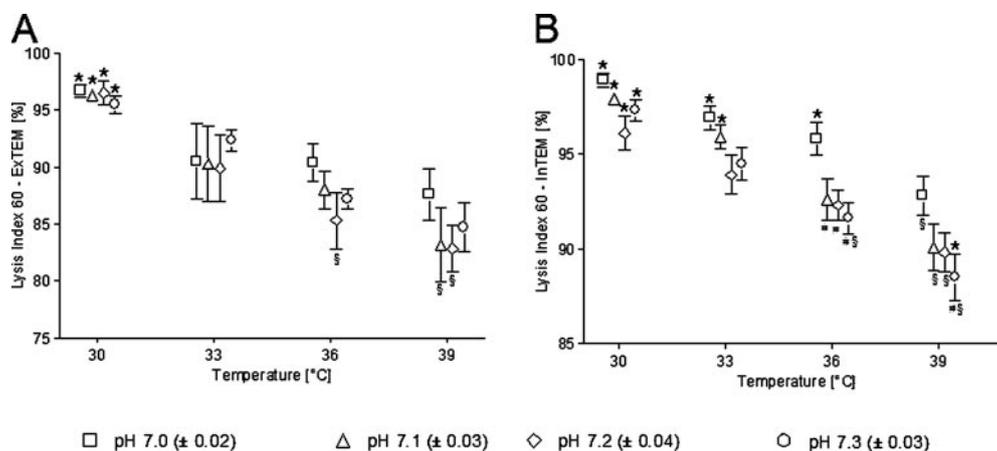


Figure 4. Effects on clot lysis of hypothermia and acidosis, and of hypothermia/acidosis combined Lysis index (LI) in ExTEM™ (A) and LI in InTEM™ (B) the different groups. *statistically significant compared with the control value at 36°C and pH 7.36 (± 0.03); #statistically significant to pH 7.36 (± 0.03) at same temperature; §statistically significant to 33°C at same pH.

already decreased significantly at 33°C. Increasing the temperature to 39°C tended to improve coagulation but this was not statistically significant.

The pH measured in the assays averaged 7.36 (± 0.03), 7.26 (± 0.04), 7.15 (± 0.03), and 7.01 (± 0.02), respectively. Acidosis by itself failed to exert significant effects on all ROTEM variables under normothermic (36°C) conditions. However, acidosis had significant effects under hypothermia. At 33°C, an additional decrease in pH significantly worsened most ROTEM-related variables. Specifically, a mild acidosis (pH 7.26 ± 0.04) combined with hypothermia of 33°C already resulted in a significantly increased CFT in both extrinsically and intrinsically activated assays (Figs. 2C and D) compared with control values at 36°C and pH 7.36 (± 0.03). A further decrease in pH and/or temperature resulted in a significantly impaired CT (Figs. 2A and B) and AA (Figs. 2E and F) as well. MCF in extrinsically activated tests was significantly impaired at 30°C and pH of 7.26 (± 0.04). A 2-way ANOVA revealed that hypothermia and a decreased pH acted synergistically to impair coagulation.

In contrast, MCF in the presence of cytochalasin D (FibTEM) was not significantly influenced by even severe hypothermia and acidosis.

Clot lysis was not increased under hypothermic, acidotic, or combined conditions but slightly increased at 39°C in the InTEM assay at pH 7.36 (± 0.03) (Fig. 4C).

No participant withdrew or had to be excluded from the study, and all samples were used for analysis.

DISCUSSION

The results of our current *in vitro* study using human whole blood and measurements by ROTEM show that initiation and propagation of coagulation, as well as the stability of a formed blood clot, are impaired by hypothermia but not by acidosis under normothermia. However, hypothermia and acidosis occurring together synergistically impair coagulation. Furthermore, MCF in the presence of cytochalasin D is not affected by hypothermia, acidosis, or their combination. Finally, fibrinolysis was not increased under hypothermia.

Different mechanisms have been considered to be responsible for the development of coagulopathy in trauma patients, and hypothermia, acidosis, and hemodilution are thought to play key roles.⁴ However, in a clinical setting, these variables cannot be assessed separately. Furthermore, standard assays of plasma coagulation do not sufficiently reflect coagulation of whole blood.

The purpose of our study was to investigate the effects of graded hypothermia and acidosis and to detect possible interactions. In contrast to a study using InTEM assays and thromboelastometry in healthy volunteers,²⁸ we did not find CFT to be significantly impaired by acidosis at normothermia. However, the authors of the latter study tested a pH as low as 6.8. Our results concerning hypothermia are consistent with prior studies using classic TEG[®] in patients undergoing cardiac surgery²⁹ or liver transplantation.³⁰

In our study, CT, CFT, and AA were impaired by a temperature of 33°C and below, in extrinsically as well as intrinsically activated assays. Since CT reflects initial thrombin generation and fibrin gel formation, it is mainly dependent on the activity of plasma coagulation factors. In contrast, CFT and AA represent the kinetics of the clot generation and propagation, i.e., further fibrin polymerization and fibrin-platelet interaction.³¹ Therefore, CFT is much more dependent on platelet count and function as well as on fibrinogen concentration than CT. Thus, impairment of CT, CFT, and AA by hypothermia likely resulted from combined impairment of plasmatic coagulation and platelet function. Of note, MCF was not impaired by hypothermia without acidosis. Together, this indicates that hypothermia *per se* slows all reactions, plasmatic and platelet-derived, but that normal clot strength is achieved eventually in the presence of sufficient concentrations of clotting factors and platelets. Obviously, however, we do not know whether this also holds true for coagulopathy in the presence of diminished clotting factors and diminished platelet count and/or function, e.g., after trauma or liver surgery.

Of interest, when combining hypothermia and acidosis, we found that the impact of acidosis is more important at a lower temperature, and that hypothermia and acidosis interact to impair coagulation variables except clot lysis. This supports the hypothesis that the coagulation enzymes' optimal pH in whole blood is approximately 7.4 or more.

MCF was impaired in both ExTEM and InTEM assays but not after inhibition of platelet cytoskeletal reorganization by cytochalasin D. Thus, the reduction of MCF evoked by hypothermia likely results, at least in part, from impaired platelet function. Fibrin polymerization apparently is not affected by acidosis and hypothermia, as reflected by normal MCF values in FibTEM assays. Thus, it is uncertain whether high normal or even supranormal fibrinogen concentrations may contribute to improvement of hemostasis in hypothermic and acidotic patients.

Neither hypothermia nor acidosis increased fibrinolysis, but we found a slight increase in the lysis index in the intrinsically activated test at pH 7.36 (± 0.03) under hyperthermic conditions. From our data, it is not possible to decide whether this results from improved platelet function/improved clot retraction³² or reflects an increase of fibrinolysis. The latter could be due to fibrinolytic enzymes being less sensitive to effects of temperature and/or acidosis than are coagulation enzymes. However, our study did not address the potential effects during activation of fibrinolysis *in vivo*, as evoked by increased tissue type plasminogen activator concentrations, a setting likely to occur in trauma patients.

A potential limitation of this investigation is that it was an *in vitro* study. In fact, hypothermia itself may cause sequestration of platelets in the liver and other changes might be observed as well. However, these studies are not feasible in humans. Furthermore, by using human whole blood *in vitro* we were able to exclude other physiological responses which by themselves would alter coagulation. In addition, by using both ExTEM and InTEM assays we assessed effects on coagulation involving factors of both the classic extrinsic and intrinsic coagulation pathways.

In summary, hypothermia and hypothermia/acidosis combined, but not acidosis alone, impair coagulation but not clot lysis, as studied by whole blood ROTEM, and the impact of acidosis increases with decreasing blood temperature. Furthermore, our data show that ROTEM can over-estimate the integrity of coagulation if performed at 37°C. Since ROTEM can be adapted very quickly for measurements at a specific temperature, it may be useful for clinical studies to assess clot formation at the patient's body temperature.

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