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Citrate Storage Affects Thrombelastograph® Analysis

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Background: **Thrombelastograph® analysis (TEG®) is used to evaluate blood coagulation. Ideally, whole blood is immediately processed. If impossible, blood may be citrated and assessed after recalcification. No data describe the effect of such treatment and storage on TEG® parameters.**

Methods: **Three studies were performed in 90 surgical patients. In 30 patients, blood was citrated (1:10, 0.129 M) and recalcified (20 μl 2 M $CaCl_2$ to 340 μl citrated blood), and TEG® was performed with native blood and after recalcification after 0, 15, and 30 min of citrate storage. In another 30 patients, TEG® was performed with citrated blood recalcified immediately and after 1–72 h storage. In a third study, thrombin–antithrombin complex, prothrombin fragment 1+2, and β-thromboglobulin were measured (using enzyme-linked immunoabsorbant assay tests) at corresponding time points. Data were compared using repeated-measures analysis of variance and *post hoc* paired tests.**

Results: **TEG® parameters were different in recalcified citrated blood compared with native blood ($P < 0.05$) and changed significantly during 30-min ($P < 0.025$) and 72-h ($P < 0.001$) citrate storage. TEG® parameters measured between and 8 h of citrate storage were stable. Thrombin–antithrombin complex and prothrombin fragment 1+2 values were not elevated in native blood. After 30 min of citrate storage a gradual thrombin activation was observed, as evidenced by increasing thrombin–antithrombin complex and prothrombin fragment 1+2 values ($P < 0.05$). -Thromboglobulin level was increased after 2 and 8 h of citrate storage ($P < 0.01$).**

Conclusions: **Analysis of native blood yields the most reliable TEG® results. Should immediate TEG® processing not be possible, citrated blood may be used if recalcified after 1–8 h. (Key words: Anticoagulants; blood; coagulation physiology; coagulation tests; time effects.)**

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THE Thrombelastograph® analysis (TEG®) is being used with increasing frequency to evaluate perioperative blood coagulation during liver transplantation,[1–3] in trauma patients,[4,5] in cardiac[6,7] and noncardiac[8] surgery, in parturients with and without preeclampsia,[9,10] and in the evaluation of effects of plasma expanders[11,12] and other drugs[13] on blood coagulation. TEG® provides information relatively quickly, within 20 to 30 min.[4,14] Ideally, whole blood is rapidly processed and TEG® is started immediately after collection.[14] If transport and handling time of native blood exceeds 6 min, however, citration and recalcification before TEG® has been recommended.[15]

Data concerning effects of citrate storage on TEG® are largely lacking. However, such knowledge is important to distinguish true alteration in blood coagulation from effects of citrate storage. Therefore, goals of this study were to compare TEG® parameters of native blood with those of citrated and immediately recalcified blood and to describe the effect of citrate storage on TEG® parameters. In addition, coagulation and platelet activation markers were assessed to explain potential changes in TEG® parameters resulting from citration and citrate storage.

Materials and Methods

With approval of the ethics committee of the University Hospital Zurich, responsible for anesthesia research, and written informed consent, 90 patients undergoing elective surgery were enrolled in three consecutive studies (30 patients in each). For all studies, the same exclusion criteria were used: known coagulation disorders, preoperative anticoagulation, current treatment with heparin, use of acetyl salicylic acid within the past 5 days, use of nonsteroidal antiinflammatory agents within the past 24 h, known renal diseases, or plasma concentration of creatinine more than 120 μM, and liver diseases or increased plasma concentration of aspartate aminotransferase ($>$ 50 U/l) or alanine aminotransferase ($>$ 50 U/l). Standard thrombosis prophylaxis with 3,000 IU low-molecular-weight heparin administered subcutaneously the evening before surgery was allowed.

A computerized TEG® coagulation analyzer (CTEG 3000; Hemoscope, Morton Grove, IL) was used. The following parameters of the TEG® trace were analyzed: reaction time (normal value, 12.0 $\pm$ 2.3 mm), the time from the start of the recording until the amplitude reaches 2 mm; coagulation time (normal value, 4.2 $\pm$ 1.6 mm), the time from the end of the reaction time until the amplitude achieves 20 mm; maximum amplitude (normal value, 63.5 $\pm$ 4.5 mm) of the TEG® tracing, represents the absolute strength of the clot; and angle α (normal value, 60.2 $\pm$ 6.7°), the angle formed by the slope of the TEG® tracing from the reaction time value to the coagulation time value. Clot lysis was represented by the percentage decrease in the area under the curve at 30 min (normal value $<$ 7.5%) and at 60 min (normal value $<$ 15%) after maximum amplitude.[16]

A series of three consecutive studies was performed.

Study I

TEG® Analysis with Native Blood and Stored Citrated Blood Recalcified after 0, 15, and 30 min. In 30 patients, 6 ml blood was collected before anesthesia induction by direct venipuncture and 18-gauge needle into a polypropylene syringe. One milliliter of native blood was filled into a tube containing 1% celite. One minute later, two aliquots of 360 μl celite-activated blood were added to two TEG® cups. One cup was used for TEG®, the other for determination of pH and ionized calcium and hemoglobin concentrations (BGElectrolytes and CO-Oximeter; Instrumentation Lab, Lexington, MA, and Electrolyte 8 Analyzer; Nova Biomedica, Waltham, MA). Another 4.5 ml blood was filled into a Vacutainer (Becton Dickinson, Basel, Switzerland), containing 0.5 ml trisodium citrate (0.129 M). One milliliter of citrated blood was filled into tubes containing 1% celite, and two aliquots of 340 μl celite-activated citrated blood were added to two TEG® cups containing 2 M $CaCl_2$, 20 μl, immediately after mixing (T_0) and 15 and 30 min later for TEG® analysis and measurement of pH and ionized calcium and hemoglobin concentrations. Blood samples were stored at room temperature.

Table 1. Demographics in Three Patient Groups, including Body Mass Index (BMI)

	Study I	Study II	Study III
n	30	30	30
Age (yr)	45 $\pm$ 15	52 $\pm$ 17	56 $\pm$ 15*
BMI (kg/m^2)	25.8 $\pm$ 4.8	25.8 $\pm$ 4.2	26.6 $\pm$ 5.0
Hemoglobin (g/dl^1)	13.3 $\pm$ 1.1	13.3 $\pm$ 1.6	14.1 $\pm$ 1.6
Prothrombin time (%)	100 $\pm$ 4	100 $\pm$ 5	101 $\pm$ 6
Platelet count (10^3 μl^1)	246 $\pm$ 52	249 $\pm$ 59	210 $\pm$ 71
Creatinine (μM)	84 $\pm$ 8	84 $\pm$ 14	93 $\pm$ 10*†
Incidence of carcinoma	2/30	3/30	2/30

Data are mean $\pm$ SD.

* $P < 0.05$ compared with study I (after Bonferroni correction).

† $P < 0.05$ compared with study II (after Bonferroni correction).

Study II

TEG® Analysis with Recalcified Citrated Blood during 72 h of Storage. After withdrawal of 15 ml blood from another 30 patients, 13.5 ml (3 $\times$ 4.5 ml) was mixed with 1.5 ml trisodium citrate (3 $\times$ 0.5 ml, 0.129 M) using three Vacutainers. TEG®, pH, and ionized calcium and hemoglobin concentrations were assessed in analogous fashion to the first study. Citrated blood was

Table 2. pH, Ionized Calcium, and Hemoglobin Concentration in Native Blood and in Recalcified Citrated Blood Immediately after Mixing and after 15 min and 30 min (T30) of Storage (Study I)

	Hb (g/dl)	pH	Ca^{2+} (mM)
Native	13.9 $\pm$ 1.5	7.38 $\pm$ 0.03	1.17 $\pm$ 0.04
T0	12.0 $\pm$ 1.2*	7.29 $\pm$ 0.03*	2.59 $\pm$ 0.22*
T15	12.0 $\pm$ 1.1*	7.31 $\pm$ 0.04*†	2.59 $\pm$ 0.19*
T30	12.0 $\pm$ 1.2*	7.34 $\pm$ 0.05*†	2.53 $\pm$ 0.18*
pT0–T30	0.81	0.01	0.12

Data are mean $\pm$ SD.

* $P < 0.05$ compared with Native.

† $P < 0.05$ compared with T0 (after Bonferroni correction).

Hb = hemoglobin concentration; Ca^{2+} = ionized calcium; Native = native blood; T0 = immediately; T15 = 15 min; T30 = 30 min; pT0–T30 = P value of overall changes of repeated measures ANOVA during storage (T0–T30).

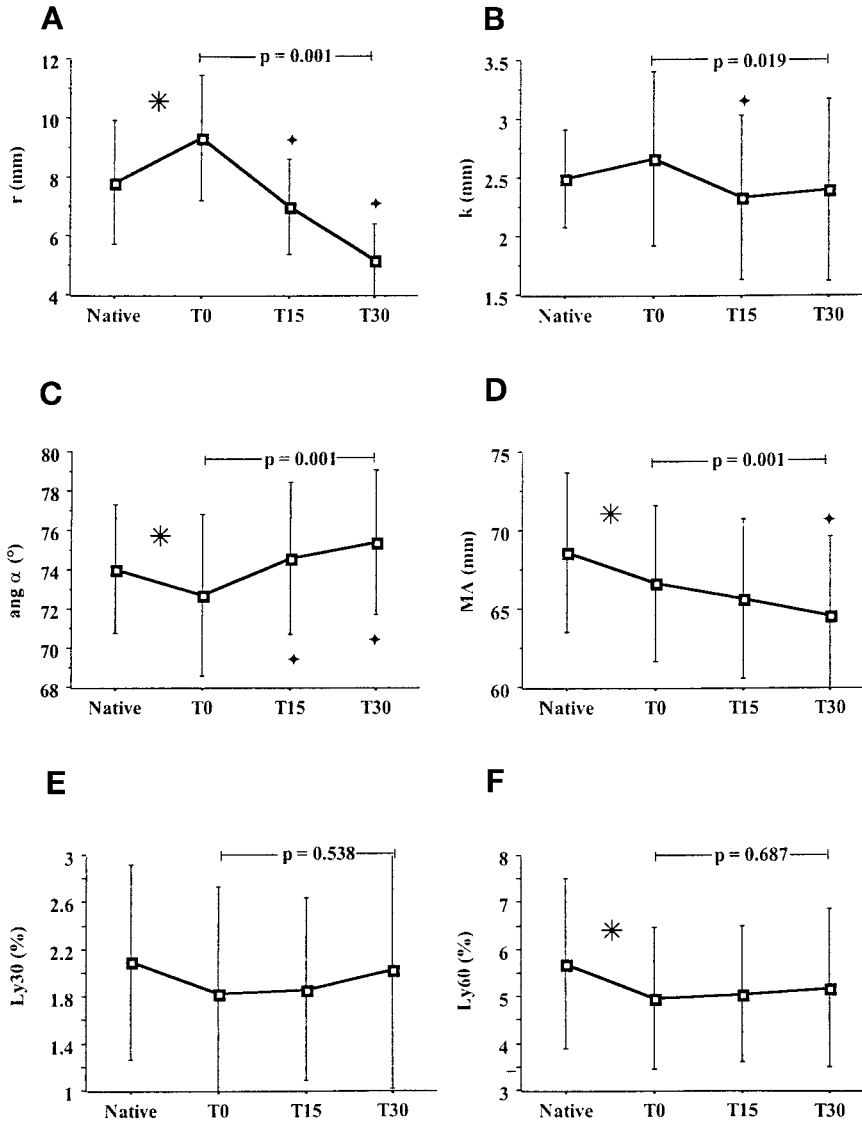

Fig. 1. Study I. Effect of 30 min of citrate storage on TEG® parameters. Reaction time (r) (*A*), coagulation time (k) (*B*), angle α (ang α) (*C*), maximum amplitude (MA) (*D*), clot lysis at 30 min (Ly30) (*E*) and clot lysis at 60 min (Ly60) (*F*) in native blood (Native) and in recalcified citrated blood immediately (T_0), 15 min, and 30 min after mixing. **P* < 0.05 compared with Native; +*P* < 0.05 compared with T_0 (after Bonferroni correction). *P* values indicated relate to overall significance of repeated-measures analysis of variance during storage.

recalcified immediately and after 1, 2, 4, 8, 24, 48, and 72 h of storage, and then TEG® analysis (with 1% celite activation) was performed. Blood samples were stored at room temperature.

Study III

Prothrombin Fragment 1+2 Assay and Thrombin–Antithrombin Complex Assay. To explain the results of study II, 16 ml blood was removed from 30 additional patients. After withdrawal, 13.5 ml (3 × 4.5 ml) was mixed with 1.5 ml trisodium citrate (3 × 0.5 ml, 0.129 M) using three Vacutainers. Native blood (0.9 ml) was mixed (9:1) with 0.1 ml of an anticoagulant cocktail (100 ml ethylene diamine tetraacetic acid, 5% [EDTA], in 0.45% NaCl containing 240 mg aminophyllin; 100,000 kallikrein inhibiting units [KIE] aprotinin [Bayer (Schweiz) AG, Pharma, Zurich, Switzerland]; and 50 mg lepirudin [Hoechst Marion Roussel AG, Zurich, Switzerland], at a pH of 5.3), put on ice and centrifuged at 2,500 rpm and at 4°C for 30 min. Plasma was separated and stored at −70°C. The remainder of native blood was used for measuring pH and ionized calcium and hemoglobin concentrations. Citrated blood was stored for a maximum period of 8 h at room temperature. Analogous to native blood, the anticoagulant

Table 3. Hemoglobin Concentration, pH, and Ionized Calcium Immediately after Mixing, after 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, and 72 h of Storage and Recalcification (Study II)

	Hb (g/dl)	pH	Ca^{2+} (mM)
T0	11.3 ± 1.3	7.25 ± 0.03	2.45 ± 0.28
T1	11.4 ± 1.3	7.27 ± 0.03*	2.41 ± 0.25
T2	11.4 ± 1.3	7.30 ± 0.04*†	2.43 ± 0.23
T4	11.3 ± 1.3	7.23 ± 0.04*†	2.44 ± 0.28
T8	11.3 ± 1.3	7.22 ± 0.04*†	2.44 ± 0.27
T24	11.2 ± 1.4†	7.07 ± 0.07*†	2.45 ± 0.27
T48	11.3 ± 1.4	6.97 ± 0.06*†	2.52 ± 0.27
T72	11.4 ± 1.4	6.96 ± 0.05*†	2.49 ± 0.25
pT0–T72	0.02	0.01	0.53

Data are mean ± SD.

* $P < 0.05$ compared with T0 (after Bonferroni correction).

† $P < 0.05$ compared with T1 (after Bonferroni correction).

Hb = hemoglobin concentration; Ca^{2+} = ionized calcium; T0 = immediately; T1 = 1 h; T2 = 2 h; T4 = 4 h; T8 = 8 h; T24 = 24 h; T48 = 48 h; T72 = 72 h; pT0–T72 denotes *P* value of overall changes of repeated measures ANOVA during storage (T0–T72).

cocktail was added immediately after mixing with citrate and after 15 and 30 min, and 1, 2, 4, and 8 h of storage.

At activation of coagulation, prothrombin fragment 1+2 (F1+2) is formed as a result of the activation of prothrombin. Thrombin exists in blood mainly as thrombin–antithrombin (TAT) complex. To evaluate coagulation activation, F1+2 and TAT concentrations were determined using enzyme-linked immunoabsorbent assay technology (Enzygnost F1+2 Micro; Enzygnost TAT Test Kit; Behring Diagnostics GmbH, Marburg, Germany). In addition, to assess platelet activation during citrate storage, β-thromboglobulin was determined in citrated blood that was stored for 0, 2, and 8 h, using enzyme-linked immunoabsorbent assay technology (Asserachrom β-TG; Behring Diagnostics GmbH).

Table 4. Comparison of TEG® Parameters in Recalcified Citrated Blood Immediately after Mixing between Studies I and II: Reaction Time, Coagulation Time, Maximum Amplitude, Angle α, Coagulation Index, Lysis at 30 min, and Lysis at 60 min

	Study 1	Study 2
r 0 (mm)	9.4 ± 2.1	9.1 ± 2.6
k 0 (mm)	2.7 ± 0.7	2.4 ± 0.5
MA 0 (mm)	66.7 ± 5.0	68.1 ± 4.1
α 0 (°)	72.8 ± 4.1	74.4 ± 3.6
Ly30 0 (%)	1.8 ± 0.9	1.6 ± 1.2
Ly60 0 (%)	5.0 ± 1.5	4.4 ± 1.8

Data are mean ± SD.

No statistically significant differences.

r 0 = reaction time; k 0 = coagulation time; MA 0 = maximum amplitude; α 0 = angle α; CI 0 = coagulation index; Ly30 0 = lysis at 30 min; Ly60 0 = lysis at 60 min.

Statistical Analysis

Data are presented as the mean ± SD. TEG® parameters of native blood and immediately recalcified citrated blood were compared using paired *t* tests (Statview 4.02; Abacus Concepts, Berkley, CA). Changes during citrate storage were analyzed by means of repeated-measures analysis of variance with Greenhouse–Geisser correction (Superanova 1.11; Abacus Concepts). If overall repeated measures analysis of variance indicated a significant change in TEG® parameters during citrate storage, data were compared with T_0 (and T_1 in study II) using paired *t* tests with Bonferroni correction. Because β-thromboglobulin data were not normally distributed, a nonparametric repeated-measures analysis of variance (Friedman test) was used, and if the results were significant, Wilcoxon signed rank tests with Bonferroni correction were performed to compare data with T_0. Unpaired *t* tests were used to compare patient characteristics among the different studies and to compare TEG® parameters in recalcified citrated blood between studies I and II. $P < 0.05$ was considered statistically significant.

Results

There were no differences among the three study groups in patient characteristics such as body mass index, hemoglobin concentration, prothrombin time, and platelet count. However, patients in study III were older than those in study I, and their creatinine concentrations were slightly higher than in patients enrolled in studies I and II (table 1).

Study I

Hemoglobin concentration of recalcified citrated blood was reduced because of citrate dilution and remained stable during 30 min of storage. pH was lower in recalcified citrated blood compared with native blood and recovered gradually during citrate storage. Ionized calcium concentration was higher after mixing with citrate and recalcification and remained stable during storage (table 2).

Reaction time was higher and angle α and maximum amplitude were lower in immediately recalcified citrated blood (at T_0) compared with native blood; coagulation time was unchanged (fig. 1). Clot lysis at 30 min was

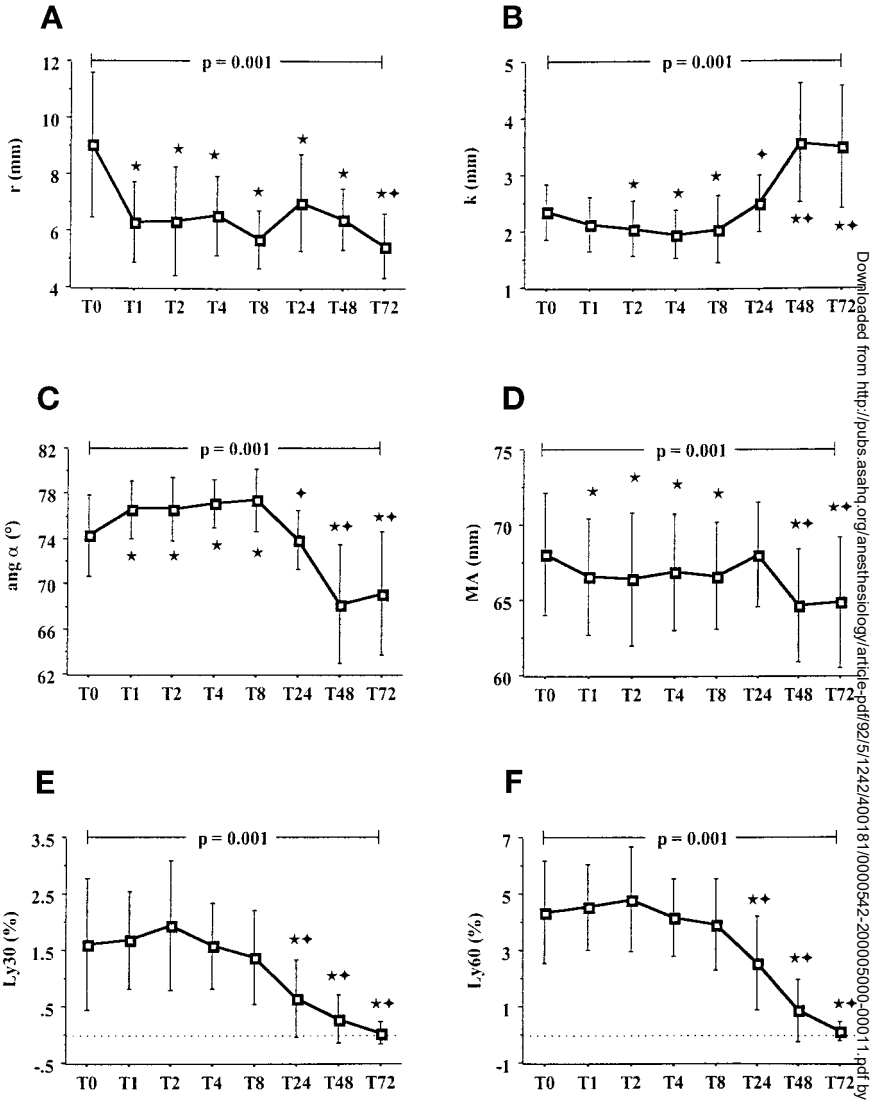

Fig. 2. Study II. Effect of 72 h of citrate storage on TEG® parameters: Reaction time (r) (*A*), coagulation time (k) (*B*), angle α (ang α) (*C*), maximum amplitude (MA) (*D*), clot lysis at 30 min (Ly30) (*E*) and clot lysis at 60 min (Ly60) (*F*) in native blood (native) and in recalcified citrated blood immediately after mixing (T_0) and after 1 (T_1), 2 (T_2), 4 (T_4), 8 (T_8), 24 (T24), 48 (T_{48}), and 72 h (T_{72}) of storage. *$P < 0.05$ compared with T_0 (after Bonferroni correction); +$P < 0.05$ compared with T_1 (after Bonferroni correction). *P* values indicated relate to overall significance of repeated-measures analysis of variance during storage (T_0–T_{72}).

unchanged, but at 60 min was slightly lower in immediately recalcified citrated blood (at T_0). During 30 min of citrate storage, significant changes were observed in reaction time, coagulation time, angle α, and maximum amplitude. Clot lysis was unaffected.

Study II

Hemoglobin concentration changed minimally, pH decreased, and ionized calcium concentration was stable during 72 h of citrate storage (table 3). There was no significant difference in TEG® parameters in recalcified citrated blood immediately after mixing between studies I and II (table 4).

All TEG® parameters changed significantly during 72 h of citrate storage (fig. 2). Reaction time decreased after 1 h and remained stable at this level until 48 h of citrate storage and decreased at 72 h of storage. Coagulation time was shorter between 2 and 8 h of citrate storage compared with immediately recalified citrated blood and was higher between 24 and 72 h of storage. Angle α increased during the first hour of storage and remained stable at this elevated level until 8 h. Angle α decreased at 24 h and was low between 48 and 72 h of citrate storage. Maximum amplitude decreased during the first hour of storage and remained stable at this level until 8 h. Between 48 and 72 h, maximum amplitude was lower compared with immediately recalified citrated blood. Clot lysis at 30 min and 60 min was unaltered during the

Table 5. Hemoglobin Concentration, pH, and Ionized Calcium in Native Blood and in Citrated Blood Immediately after, 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h (Study III)

	Hb (g/dl)	pH	Ca^{2+} (mM)
Native	14.4 ± 1.6	7.41 ± 0.02	1.15 ± 0.04
T0	13.1 ± 1.5*	7.23 ± 0.02*	0.04 ± 0.01*
T15	13.1 ± 1.5*	7.25 ± 0.04*†	0.03 ± 0.01*
T30	13.1 ± 1.5*	7.24 ± 0.05*	0.03 ± 0.01*
T1	13.1 ± 1.5*	7.24 ± 0.03*	0.03 ± 0.01*
T2	13.1 ± 1.5*	7.25 ± 0.04*†	0.03 ± 0.01*
T4	13.1 ± 1.5*	7.20 ± 0.04*†	0.03 ± 0.01*
T8	13.1 ± 1.5*	7.16 ± 0.04*†	0.03 ± 0.01*
p (T0–T8)	0.01	0.01	0.01

Data are mean ± SD.

* $P < 0.05$ compared with Native.

† $P < 0.05$ compared with T0 (after Bonferroni correction).

Hb = hemoglobin concentration; Ca^{2+} = ionized calcium; Native = native blood; T0 = immediately; T15 = 15 min; T30 = 30 min; T1 = 1 h; T2 = 2 h; T4 = 4 h; T8 = 8 h; pT0–T8 denotes *P* value of overall changes of repeated measures ANOVA during storage (T0–T8).

first 8 h of storage and decreased between 24 and 72 h of citrate storage (fig. 2).

Study III

Hemoglobin and ionized calcium concentrations decreased because of citrate administration and remained at this decreased level during 8 h of storage. Also, pH decreased because of citrate administration and decreased further between 4 and 8 h of storage (table 5).

Both TAT and F1+2 levels were unaffected by citrate administration. However, TAT and F1+2 levels increased during citrate storage. After 1 h of storage, the TAT level was higher compared with native blood and citrated blood without storage. Already at 30 min, F1+2 level was higher than before storage. Between 1 and 8 h, TAT and F1+2 levels were higher than in native blood or citrated blood without storage. Also, β-thromboglobulin level increased during citrate storage (fig. 3).

Discussion

We demonstrated that TEG® blood coagulation parameters in recalcified citrated blood differ from those in native blood and change significantly during 30–60 min of storage but remain stable between 1 and 8 h. After 24 h, coagulation parameters were largely different from those measured after 1 h of storage. Changes in TEG® blood coagulation parameters during storage may, in

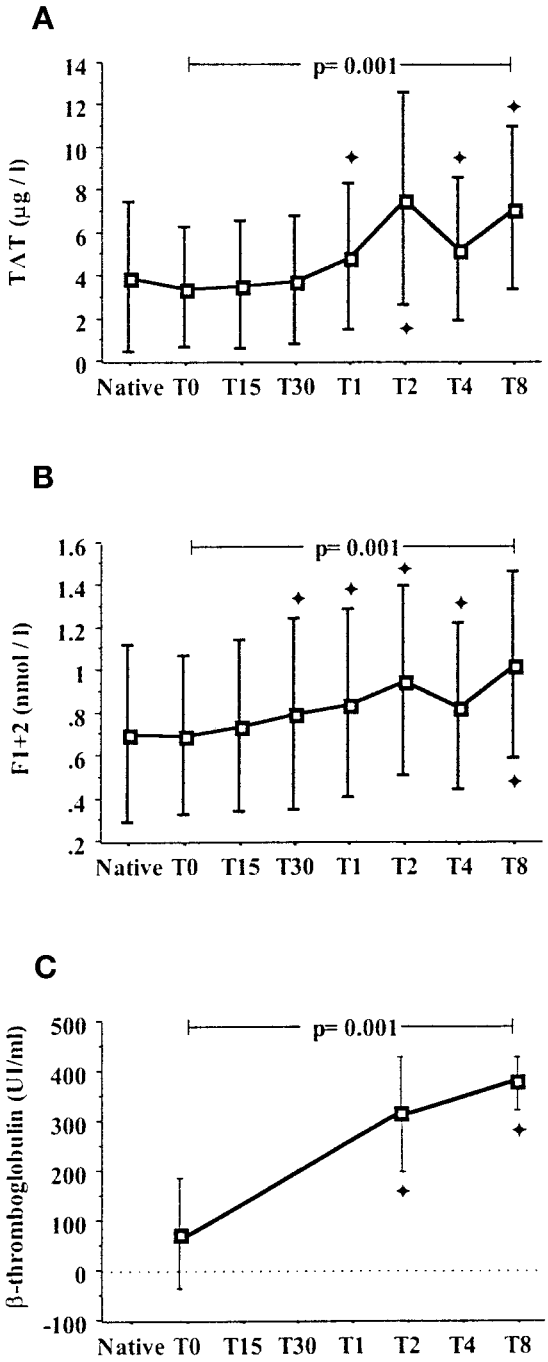

Fig. 3. Study III. Thrombin–antithrombin (TAT) complex (*A*), prothrombin fragment 1+2 (F1+2) (*B*), and β-thromboglobulin (*C*) concentrations in native blood (native) and in citrated blood immediately after mixing (T_0) and after 15 min (T_{15}), 30 min (T_{30}), 1 h (T_1), 2 h (T_2), 4 h (T_4), and 8 h (T_8) of storage. *$P < 0.05$ compared with Native, +$P < 0.05$ compared with T_0 (after Bonferroni correction). *P* values indicated relate to overall significance of repeated-measures analysis of variance during storage (T_0–T_8).

part, be explained by activation of coagulation and platelets in citrated blood.

Ideally, blood coagulation is evaluated by TEG® immediately (< 6 min) after sampling.[14,16] Alternatively, collection of citrated blood is recommended with recalcification for TEG® assessment.[15] Interestingly, no systematic studies are available about the effect of citrate recalcification and citrate storage on TEG® parameters. For all situations in which immediate TEG® determination is not practically feasible, for example, in operating rooms, intensive care units, and emergency rooms without 24-h availability of a TEG® analyzer; for various research purposes in which multiple TEG® analyses must be performed simultaneously; or for out-of-hospital research, clear operating procedures are important to distinguish between true alterations in blood coagulation and artifacts introduced by inconsistent TEG® methodology. Such artifacts may mislead blood coagulation assessment in the direction of hypo- or hypercoagulability, although the difference to the true blood coagulation status may not be diagnostic by itself.

Immediate recalcification of citrated blood results in a mild form of hypocoagulability, as evidenced by an increase in reaction time a decrease in angle α, and a reduced maximum amplitude (fig. 1). The lower maximum amplitude may, in part, be explained by an altered platelet reactivity, as described previously by Bar *et al.*[17] The prolongation of TEG® parameters mainly influenced by coagulation factor activity, such as reaction time, coagulation time, and angle α, however, is more difficult to explain. As shown in a previous study,[18] an increased reaction time can be caused by increased thrombin generation. However, we did not observe any thrombin generation in the immediately recalcified citrated blood (fig. 3).

A progressive acceleration of blood coagulation occurred during 30 min (study I, fig. 1) to 60 min (study II, fig. 2) citrate storage, with decreases in reaction time and coagulation time and an increase in angle α. This may be explained by an activation of the coagulation cascade, as shown by thrombin generation evidenced by increasing levels of F1+2 and TAT after 30–60 min citrate storage (fig. 3). This is conceptually substantiated by the finding that the TEG® parameters affected most are those reflecting coagulation factor activity.

Maximum amplitude decreased during citrate storage between 30 (study I, fig. 1) and 60 min (study II, fig. 2). This may be related to platelet activation, as evidenced by increased levels of β-thromboglobulin (fig. 3).[19–22] Platelets having released significant quantities of β-thromboglobulin during citrate storage may have decreased ability to adequately contribute to blood coagulation during subsequent TEG® measurement. This may result in a reduced clot strength, with a compromised maximum amplitude.

Elevated calcium concentrations were reached after recalcification (tables 2 and 3). Coagulation testing in citrated blood is routinely performed at elevated calcium concentrations to avoid residual citrate effects. Also thrombin generation is nonlinearly affected by calcium concentration. At very low calcium concentrations thrombin generation is reduced; as calcium concentration increases, thrombin generation increases and soon reaches a plateau.[23] Compromised blood coagulation because of elevated calcium concentration is therefore relatively unlikely. Formal studies may clarify this issue for TEG® in the future.

The finding that citration could not prevent completely the activation of coagulation and platelets is remarkable. Although sodium citrate has been shown to be an adequate anticoagulant for measuring coagulation activation markers for clinical purposes,[22] it is not sufficient to completely suppress thrombin generation *in vitro*. It has been shown that minimal amounts of activation markers, as generated by the venipuncture itself, may be still detectable in citrated plasma.[24] Therefore we determined TAT, F1+2, and β-thromboglobulin levels after citrate storage only after irreversibly blocking the coagulation with different anticoagulants. Whereas most clinical assays that include activated partial prothromin time, prothrombin time, and thrombin time are insensitive to minor thrombin generation during citrate storage,[24,25] this study shows that TEG® analysis may be sensitive to procoagulant alterations.

In conclusion, TEG® analysis in native blood yields the most reliable results, provided the TEG® is processed within 6 min of sampling. If this is impossible, citrated blood may be collected and TEG® measurements performed after recalcification. To achieve reproducible results, citrated blood should be stored at least 1 h and a maximum of 8 h.

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