

# Asphyxia by Drowning Induces Massive Bleeding Due To Hyperfibrinolytic Disseminated Intravascular Coagulation

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**Objective:** To date, no study has systematically investigated the impact of drowning-induced asphyxia on hemostasis. Our objective was to test the hypothesis that asphyxia induces bleeding by hyperfibrinolytic disseminated intravascular coagulation.

**Design:** Observational study.

**Setting:** A 2,100-bed tertiary care facility in Vienna, Austria, Europe.

**Patients:** All cases of drowning-induced asphyxia ( $n = 49$ ) were compared with other patients with cardiopulmonary resuscitation ( $n = 116$ ) and to patients with acute promyelocytic leukemia ( $n = 83$ ). Six drowning victims were investigated prospectively. To study the mechanism, a forearm-ischemia model was used in 20 volunteers to investigate whether hypoxia releases tissue plasminogen activator.

**Interventions:** None.

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**Measurements and Main Results:** Eighty percent of patients with drowning-induced asphyxia developed overt disseminated intravascular coagulation within 24 hours. When compared with nondrowning cardiac arrest patients, drowning patients had a 13 times higher prevalence of overt disseminated intravascular coagulation at admission (55% vs 4%;  $p < 0.001$ ). Despite comparable disseminated intravascular coagulation scores, acute promyelocytic leukemia patients had higher fibrinogen but lower d-dimer levels and platelet counts than drowning patients ( $p < 0.001$ ). Drowning victims had a three-fold longer activated partial thromboplastin time (124 s;  $p < 0.001$ ) than both nondrowning cardiac arrest and acute promyelocytic leukemia patients. Hyperfibrinolysis was reflected by up to 1,000-fold increased d-dimer levels, greater than 5-fold elevated plasmin antiplasmin levels, and a complete absence of thrombelastometric clotting patterns, which was reversed by antifibrinolytics and heparinase. Thirty minutes of forearm-ischemia increased tissue plasminogen activator 31-fold ( $p < 0.001$ ).

**Conclusions:** The vast majority of drowning patients develops overt hyperfibrinolytic disseminated intravascular coagulation, partly caused by hypoxia induced tissue plasminogen activator release. Antifibrinolytics and heparinase partially reverse the abnormal clotting patterns. Severe activated partial thromboplastin time prolongation may be a marker of combined hyperfibrinolytic afibrinogenemia and autoheparinization in drowning-related asphyxia. (*Crit Care Med* 2015; 43:2394–2402)

**Key Words:** asphyxia disseminated intravascular coagulation; bleeding; cardiac arrest; drowning

Drowning leads to respiratory insufficiency and ensuing asphyxia to cardiac arrest (CA). While resuscitating individual drowning victims, we observed clinically profuse bleeding already at admission of these patients. Interested in the mechanism, we searched for available published data, but were unable to find any. Despite a couple of case reports (1, 2) describing similar findings more than three decades ago, to date, no study has investigated coagulopathy

following drowning. We hypothesized hyperfibrinolytic disseminated intravascular coagulation (DIC) as an underlying mechanism of bleeding.

Hyperfibrinolytic DIC is a bleeding-dominant coagulopathy, where uncontrolled fibrinolysis with massive fibrinogen consumption impairs clotting.

Conditions with a risk of hyperfibrinolysis are heterogeneous and include acute promyelocytic leukemia (APL) (3–6), other malignant neoplasms (7–9), fulminant sepsis (10) and traumatic shock (11). In APL, hyperfibrinolytic DIC occurs in up to 70% and is a main prognostic determinant of survival with a DIC score of greater than or equal to 6 predicting early hemorrhagic death (12). Likewise, hyperfibrinolytic coagulopathy was shown to predict mortality in trauma (13–15).

However, pathophysiologic mechanisms causing a fibrinolytic DIC are not fully elucidated and likely vary dependent on the underlying pathology.

The current study investigated specific coagulation patterns following drowning-related CA and aimed to elucidate possible underlying mechanisms of drowning-induced DIC.

## MATERIALS AND METHODS

The study protocols were approved by the Ethics Committee of the Medical University of Vienna and conducted in accordance with Declaration of Helsinki. Written informed consent was obtained from all prospectively investigated subjects who were able to give consent. Otherwise, a waiver was obtained, and patients were informed about their study participation in case of regaining consciousness.

### Overview

Coagulation patterns of all patients with drowning-related CA admitted to the University Hospital of Vienna from January 1996 to July 2014 were analyzed. Patients with nondrowning CA ( $n = 116$ ) and patients with APL ( $n = 83$ ) were used as controls. For DIC calculation, the International Society on Thrombosis and Haemostasis (ISTH)-DIC score was applied (16).

Patients with anticoagulants intake, thrombolysis, or heparin therapy were excluded from analysis. Patient charts were manually reviewed by two investigators (M.S., B.J.) to determine the frequency of clinical bleeding in drowning patients. Neurologic outcome (at 1 and 6 mo) of drowning and nondrowning CA survivors was assessed by Cerebral Performance Category (CPC; CPC 1–2: good outcome and CPC 3–5: poor outcome) (17). Patients with CA with a pH less than or equal to 7.0 were compared in a subgroup analysis.

Six drowning victims were investigated prospectively by thromboelastometry (rotational thromboelastometric analysis [ROTEM]), coagulation studies, and measurements of tissue plasminogen activator (tPA) levels, plasmin antiplasmin complexes, syndecan-1 (as marker of endothelial damage), and tryptase and heparan sulfate levels (to test for endogenous heparin-like substances).

To investigate mechanisms of coagulopathy in drowning patients in more detail, we 1) analyzed the potency

of antifibrinolytics (tranexamic acid [tranexamic acid [Cyklokapon] is not labeled for the use in patients with cardiac arrest] and aprotinin) and heparinase to restore clotting in blood samples obtained from drowning patients, 2) induced hyperfibrinolysis by mixing plasma obtained from three drowning patients to RBCs from healthy volunteers and restored clotting by adding antifibrinolytics, 3) established a forearm-ischemia/hypothermia model in 20 healthy volunteers (to test the impact of hypoperfusion and hypothermia on coagulation patterns and plasma tPA levels), and 4) conducted in vitro experiments on whole-blood samples (obtained from 10 volunteers) using commercially available lactic acid and recombinant tPA (r-tPA) to determine the impact of acidosis and hyperfibrinolysis on the coagulation profile. Finally, we investigated the coagulation profile of 10 patients with severe diabetic ketoacidosis to examine whether endogenous acidosis alters coagulation patterns,

**Drowning.** Drowning is defined as the process of experiencing respiratory impairment from sub/immersion in liquid according to the definition by the World Health Organization (18).

**DIC Score.** The ISTH-DIC scoring system (16) proposes a validated diagnostic algorithm based on four coagulation parameters using a point-scale from 0 to 8 (with a value  $\geq 5$  defining overt DIC): fibrinogen (180–380 mg/dL;  $< 100$  mg/dL = 1), d-dimer ( $< 0.4$   $\mu\text{g/mL}$  = 0,  $0.4$ – $4$   $\mu\text{g/mL}$  = 2, and  $> 4$   $\mu\text{g/mL}$  = 3), prothrombin time (PT;  $> 70\%$  = 0,  $40$ – $70\%$  = 1, and  $< 40\%$  = 2), and platelets ( $150$ – $350 \times 10^9/L$ ;  $> 100 \times 10^9/L$  = 0,  $100$ – $50 \times 10^9/L$  = 1,  $< 50 \times 10^9/L$  = 2) (19).

### Laboratory Methods

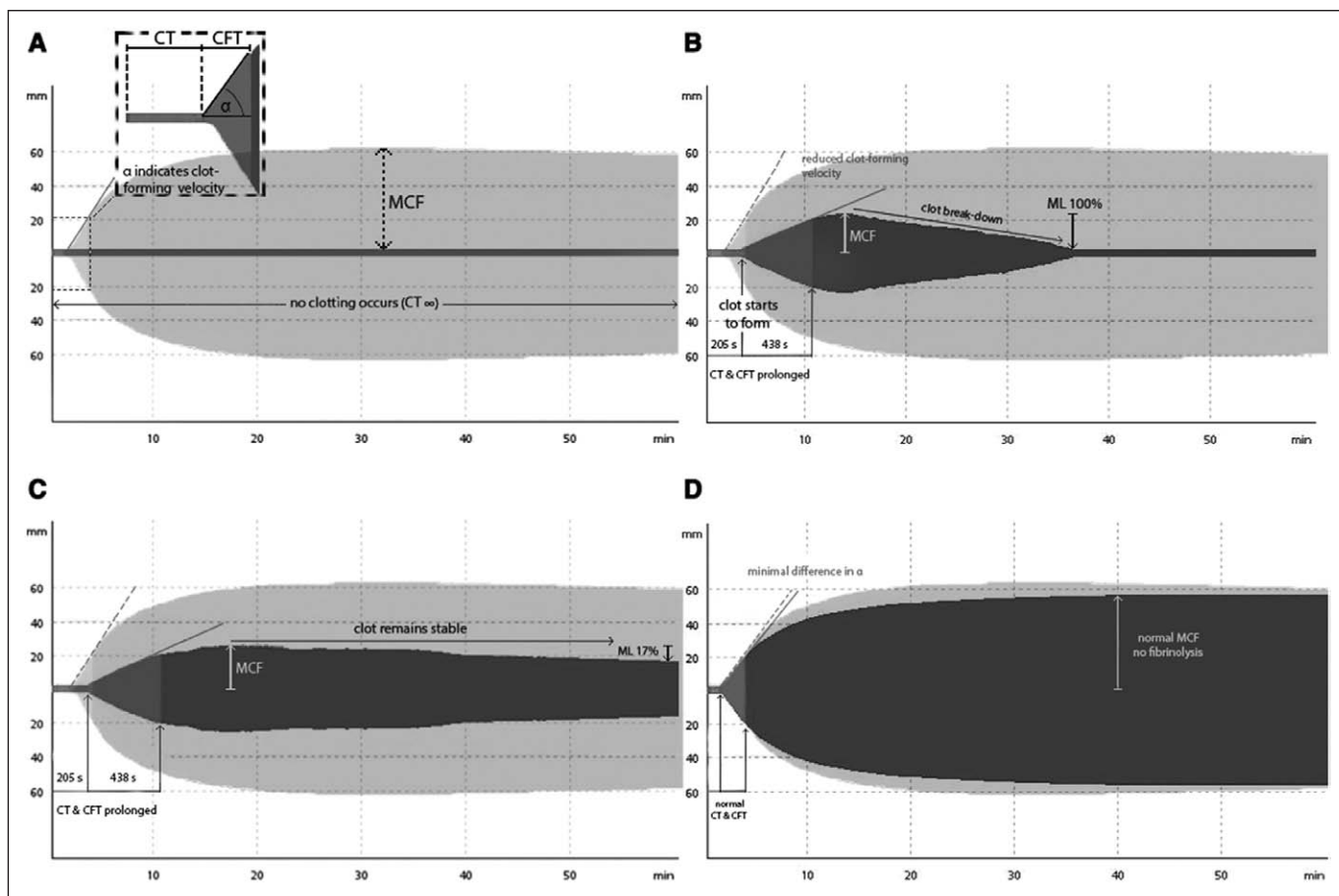
Global coagulation studies were done as described previously (20). Fibrinogen levels less than 100 mg/dL were defined as hypofibrinogenemia (according to the cutoff value suggested by the ISTH), levels less than 50 mg/dL as afibrinogenemia. The **supplemental appendix** (Supplemental Digital Content 1, <http://links.lww.com/CCM/B400>) provides details on enzyme-linked immunosorbent assay measurements and in vitro experiments.

### ROTEM

Thrombelastometry was assessed in 3.8% sodium-citrate blood samples using ROTEM (TEM International GmbH, Munchen, Germany), as described previously (21). The following tests were applied: nonactivated test using a recalcifying starting reagent, tissue factor activates extrinsic hemostasis, kaolin activates contact phase, aprotinin abolishes lysis, and heparinase inhibits heparin effect. Parameters analyzed are shown in **Figure 1**.

### Statistical Analysis

Statistical calculations were performed using SPSS Statistical Software 20.0 (IBM, Armonk, NY). Mean ( $\pm$  SD) or medians and interquartile range are provided for descriptive analysis, and nonparametric tests were used for reasons of robustness. Categorical data are presented as absolute and relative frequencies. Nonparametric tests ( $U$  test and chi-square test) were performed as appropriate. Correlations were calculated using the Spearman rank correlation test. Multivariate logistic regression



**Figure 1.** Tissue factor activates extrinsic hemostasis (EXTEM) traces from admission to 6 hr after arrival of a drowning victim. A reference rotational thrombelastometric analysis trace (25% opacity) is used as overlay to visualize differences from normal clotting. Parameters analyzed are clotting time (CT, s: time from adding starting reagent until clot begins to form; range, EXTEM [35–80 s], kaolin activates contact phase [INTEM, 100–240 s]), clot formation time (CFT, s: time from CT until a trace amplitude of 20 mm is reached; range: EXTEM [36–160 s], INTEM [35–110 s]), alpha angle ( $\alpha$ , °: angle of tangent at 2-mm amplitude: kinetic of clot formation), maximum clot firmness (MCF, mm: maximum trace amplitude, range: EXTEM, INTEM: [53–72 mm]) and maximum lysis (ML, %, difference between MCF and lowest trace amplitude in %; range: EXTEM, INTEM: [ $< 15\%$ ]). **A**, Admission, **(B)** 100 min, **(C)** 180 min, and **(D)** 360 min after first presentation. Hyperfibrinolysis progressively resolved and MCF increased over time, after tranexamic acid (1,000 mg) and fibrinogen (4,000 mg) had been given IV.

analysis identified independent predictors of activated partial thromboplastin time (APTT). The Kaplan-Meier method was used to describe survival, and the log-rank was performed for group comparisons. A two-tailed  $p$  value of less than 0.05 was considered significant.

## RESULTS

**Table 1** shows admission characteristics of drowning and nondrowning CA patients. Profuse bleeding was reported in more than 40% of all drowning patient records. Accordingly, all prospectively investigated drowning patients presented with pronounced bleeding from various sites, accompanied by a 100% fibrinolysis in ROTEM analysis (Fig. 1; and **Supplemental Figs. 1–4**, Supplemental Digital Content 2, <http://links.lww.com/CCM/B401>).

### DIC Score: Drowning Patients Versus Nondrowning CA Patients

Overt DIC was 13 times more frequent in drowning patients than in nondrowning CA (55% vs 4%;  $p < 0.001$ ) and was present in

80% of drowning patients surviving until day 2. Forty-nine percent of drowning patients presented with a platelet count less than  $100 \times 10^9/L$  (vs 9% of nondrowning CA;  $p < 0.001$ ), resulting in an overall 45% lower platelet count at admission. Drowning victims had 20-fold higher d-dimer (up to 1,200-fold the upper limit) and 69% lower fibrinogen levels: 51% of drowning patients had hypofibrinogenemia (vs 5% of nondrowning CA patients;  $p < 0.001$ ), and 31% presented with afibrinogenemia (vs 3%  $p < 0.001$ ). Drowning patients with overt DIC had afibrinogenemia in 52% (vs 20% [ $n = 1/5$ ] in nondrowning CA) and hypofibrinogenemia in 70% (vs 60% [ $n = 3/5$ ] in nondrowning CA). Median PT was slightly lower in drowning victims (71% vs 82%;  $p = 0.07$ ) although 47% had a PT less than 70% (vs 18% of nondrowning CA patients) (**Fig. 2**).

Comparing subgroups with a pH less than or equal to 7.0, differences in admission-DIC score remained significant ( $p < 0.001$ ) with lower platelet counts ( $110 \pm 66$  vs  $165 \pm 97 \times 10^9/L$ ;  $p = 0.021$ ), lower fibrinogen ( $114 \pm 74$  vs  $287 \pm 218$  g/dL;  $p = 0.011$ ), higher d-dimer levels ( $253 \pm 222$  vs  $101 \pm 109$   $\mu\text{g/mL}$ ;  $p = 0.071$ ), and a lower PT ( $76 \pm 29$  vs  $91 \pm 23\%$ ;  $p = 0.073$ ) in

**TABLE 1. Admission Characteristics of Drowning Patients Compared With Patients With Nondrowning Cardiac Arrest**

	Nondrowning (n = 116)	Drowning (n = 49)	p
Male sex	84 (72)	29 (60)	0.15
Age, yr	56 (53–59)	34 (28–40)	< 0.001 <sup>a</sup>
Cardiopulmonary resuscitation duration, min	25 (21–28)	46 (31–61)	0.013 <sup>a</sup>
Initial shockable rhythm, n (%)	66 (57)	13 (27)	< 0.001 <sup>a</sup>
Body temperature, °C	32 (29–35)	26 (24–29)	< 0.001 <sup>a</sup>
APTT, s	40 (35–78)	124 (63–180)	< 0.001 <sup>a</sup>
APTT > 180 s, n (%)	2 (1.7)	22 (45)	< 0.001
Prothrombin time (%) <sup>b</sup>	82 (65–100)	71 (54–88)	0.07
Platelets, ×10 <sup>9</sup> /L	201 (152–249)	111 (63–180)	< 0.001 <sup>a</sup>
Fibrinogen, mg/dL	343 (267–403)	107 (50–185)	< 0.001 <sup>a</sup>
Fibrinogen < 50 mg/dL, n (%)	3 (2.6)	15 (31)	< 0.001 <sup>a</sup>
D-dimer, µg/mL	7 (3–16)	146 (52–388)	< 0.001 <sup>a</sup>
Disseminated intravascular coagulation score ≥ 5, n (%)	5 (4)	27 (55)	< 0.001 <sup>a</sup>
pH	7.13 (7.1–7.2)	6.7 (6.6–6.8)	< 0.001 <sup>a</sup>
Lactate, mmol/L	10 (9–11)	21 (20–25)	< 0.001 <sup>a</sup>
PaCO <sub>2</sub> , mm Hg	49 (32–65)	55 (29–78)	0.39
Base excess, -, mmol/L	n.a.	22 (19–27)	n.a.
Lactate dehydrogenase, U/L	n.a.	1,378	n.a.
Free hemoglobin, mg/dL	34	186	< 0.001 <sup>a</sup>

APTT = activated partial thromboplastin time, n.a. = not assessed.

<sup>a</sup>Statistically significant.

<sup>b</sup>Prothrombin time was measured using Normotest.

p values are provided for descriptive purpose only. Data are expressed as patient numbers (n) and medians (interquartile range) or n (%).Mann-Whitney U test and chi-square test were used for group comparisons.

drowning patients. The DIC score correlated with cardiopulmonary resuscitation (CPR) duration only in nondrowning CA patients ( $r = 0.38$ ;  $p = 0.015$  vs  $r = 0.03$ ;  $p = 0.26$ ).

### Drowning Versus APL Patients

Overt DIC was also highly prevalent in APL (63%). Both groups presented with a median DIC score of 5 ( $p = 0.78$ ). Patients with APL had 77% lower platelet counts and 80% lower d-dimer levels ( $p < 0.001$ ), but 50% higher fibrinogen levels ( $p = 0.012$ ), and similar PT. Thrombocytopenia contributed significantly more (1.68 vs 0.61 points;  $p < 0.001$ ) and hypofibrinogenemia contributed significantly less (0.35 vs 0.63 points;  $p = 0.03$ ) to the DIC score in APL (Fig. 3).

Prolonged APTT is a characteristic feature of drowning patients (Fig. 2) and correlates with markers of hyperfibrinolysis:

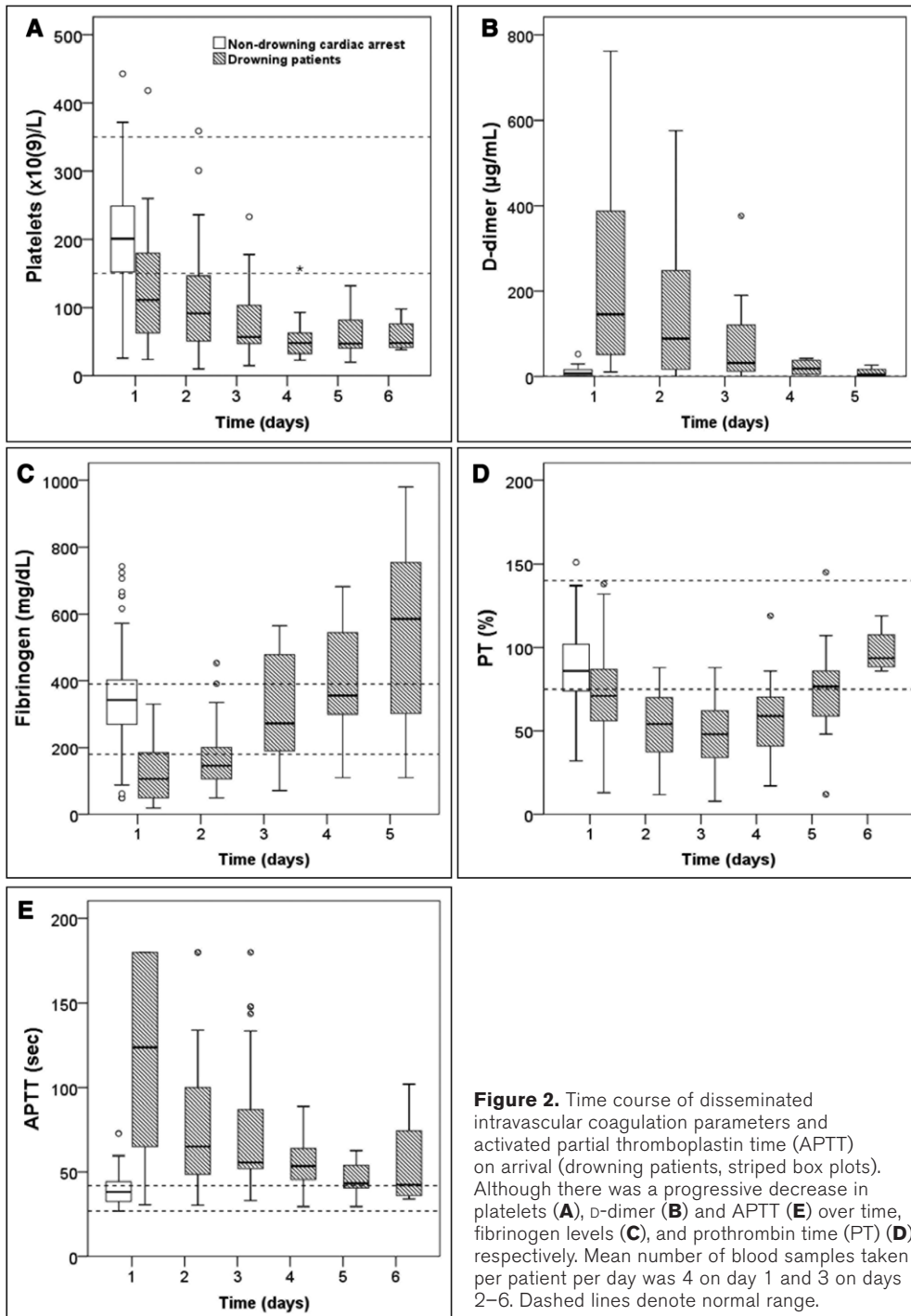
Overall, 45% ( $n = 22$ ) of drowning patients presented with APTT greater than 180 seconds (vs two nondrowning CA patients). Median APTT (124 s; 63–180) was three-fold longer in drowning victims than in nondrowning CA

(40 s; 35–78) and APL (34 s; 27–41). Seventy-three percent of drowning patients with afibrinogenemia ( $n = 11/15$ ) and 70% of drowning patients with overt DIC ( $n = 19/27$ ) had a APTT greater than 180 seconds compared with two out of five and one out of three nondrowning CA patients with DIC greater than or equal to 5 and fibrinogen less than 50 mg/dL, respectively. Patients with APL had neither afibrinogenemia nor APTT greater than 180 seconds. In drowning patients, APTT correlated strongest with d-dimer ( $r = 0.55$ ), fibrinogen ( $r = -0.58$ ), pH ( $r = -0.61$ ), and the DIC score ( $r = 0.61$ ) (all  $p < 0.001$ ). In multiple regression analysis with APTT as dependent variable, DIC score, d-dimer, and pH significantly predicted APTT (all  $p < 0.05$ ), explaining 59% of its variability ( $R^2 = 0.59$ ). No such correlations were found in APL or nondrowning CA.

### Survival of Drowning Patients Versus Nondrowning CA Patients

The majority of drowning patients died (92%;  $n = 45$ ). Non-survivors were significantly older (36 vs 14 yr,  $p = 0.025$ ), had





**Figure 2.** Time course of disseminated intravascular coagulation parameters and activated partial thromboplastin time (APTT) on arrival (drowning patients, striped box plots). Although there was a progressive decrease in platelets (A), d-dimer (B) and APTT (E) over time, fibrinogen levels (C), and prothrombin time (PT) (D) respectively. Mean number of blood samples taken per patient per day was 4 on day 1 and 3 on days 2–6. Dashed lines denote normal range.

a worse base excess ( $-24 \pm 7$  vs  $-14 \pm 3$  mmol/L,  $p = 0.016$ ), a higher d-dimer level ( $240 \pm 215$  vs  $38 \pm 22$  µg/mL,  $p = 0.13$ ), and a longer APTT ( $127 \pm 57$  vs  $93 \pm 59$  s;  $p = 0.39$ ) on arrival. CPR times were similar between both groups (42 vs 46 min;  $p = 0.95$ ). All four drowning survivors had a good neurologic outcome (CPC 1–2) at 1 month.

In nondrowning CA, 42% of patients ( $n = 49$ ) survived to hospital discharge (39% survival rate at 6 months), with a good 1-month neurologic outcome in 63% ( $n = 31$ ) (Fig. 4).

## Results of In Vitro Experiments, Forearm-Hypoxemia Model, and Findings in Prospectively Investigated Drowning Patients

More detailed results are available with the supplemental appendix (Supplemental Digital Content 1, <http://links.lww.com/CCM/B400>)

### Forearm-Ischemia Model.

Thirty minutes of arterial forearm-ischemia increased tPA levels 31-fold (to 6.8 ng/mL) but did not induce hyperfibrinolysis in ROTEM and had no influence on global coagulation studies. Hypothermia had no effect on coagulation patterns.

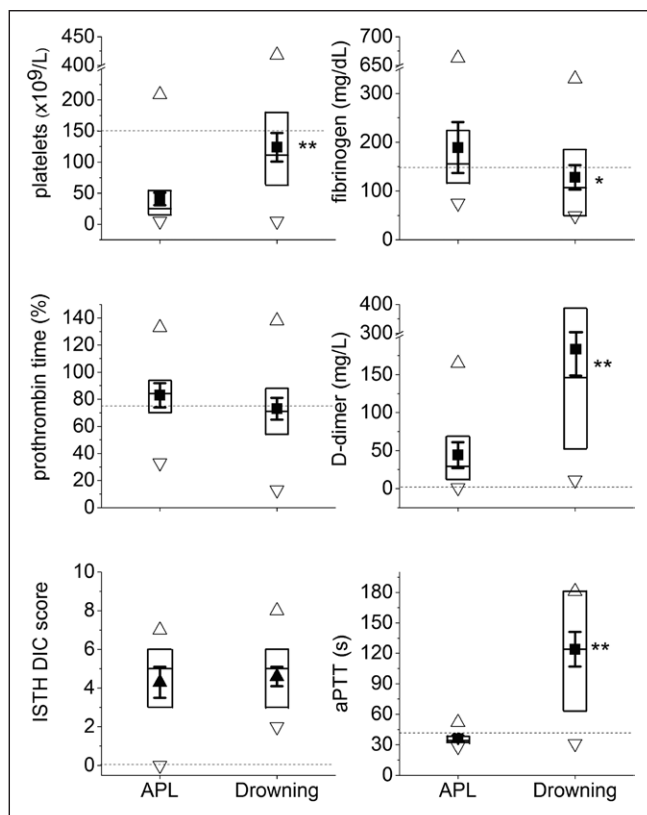
**Endogenous Acidosis.** All patients with ketoacidosis had normal global coagulation studies.

**Experimental Acidosis and Hyperfibrinolysis.** Acidification of whole-blood samples (to a pH of 6.3) with lactic acid prolonged APTT by 63% and clotting time (CT) by 49% but had no impact on PT, fibrinogen, and d-dimer. A concentration of 10 ng/mL of r-tPA were sufficient to induce fibrinolysis in ROTEM, but much higher doses ( $> 6$  µg/mL) were necessary to prolong the APTT (to a maximum of 68 s). PT was not affected by r-tPA.

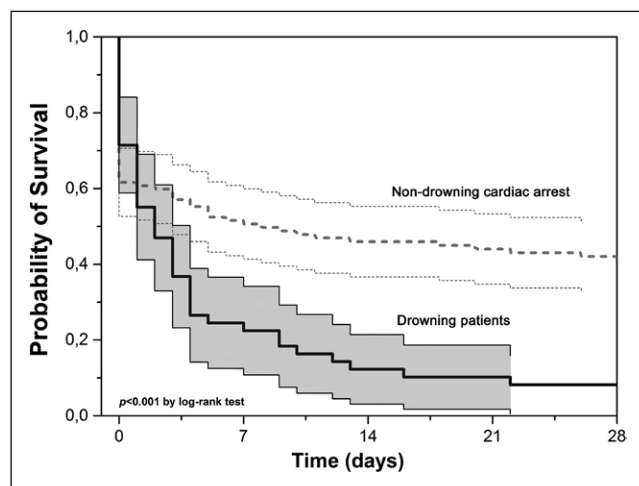
**Impact of Antifibrinolytics and Heparinase on Coagulation Patterns of Drowning Patients.** Adding heparinase to admission-blood samples of drowning patients ( $n = 3$ ) shortened the APTT from greater than 180 to  $111 \pm 36$  seconds, and both heparinase

and aprotinin reduced the CT from greater than 5,400 seconds to  $598 \pm 121$  and  $544 \pm 87$  seconds, respectively (Fig. 5).

**Hyperfibrinolysis Was Established by Mixing Plasma From Drowning Patients ( $n = 3$ ) With Volunteers' RBCs.** The addition of patient plasma to RBCs of volunteers completely abolished clotting ( $CT > 5,400$  s) in ROTEM analysis. This was partially reversed by adding tranexamic acid and aprotinin, which shortened the CT from greater than 5,400 to  $1,045 \pm 75$  and  $625 \pm 106$  seconds, respectively (Fig. 6).



**Figure 3.** Comparison of platelets, fibrinogen, prothrombin time (PT), d-dimer, disseminated intravascular coagulation (DIC) score, and activated partial thromboplastin time (APTT) between patients with acute promyelocytic leukemia (APL) and drowning patients. Data are mean (squares), 95% CIs (error bars), medians (lines), upper and lower quartiles (boxes), and minimum and maximum values (triangles). Dashed lines indicate the normal upper limits. ISTH = International Society on Thrombosis and Haemostasis.



**Figure 4.** Kaplan-Meier curves (95% CIs) showing the probability of survival of drowning patients and patients with nondrowning CA.

#### Enzyme-Linked Immunosorbent Assay Measurements.

Drowning patients had 100-fold higher tPA levels ( $24 \pm 6$  ng/mL) and greater than 55-fold higher plasmin antiplasmin levels ( $39,381 \pm 19,268$   $\mu$ g/L) than volunteers at baseline. Syndecan-1, tryptase, and heparan sulfate levels were in the normal range

(Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/B400>).

Figure 1 shows normalization of clotting signature in a drowning patient after intravenous administration of tranexamic acid (1,000 mg) and fibrinogen (4,000 mg).

## DISCUSSION

This is the first systematic investigation of coagulopathy in drowning. The high prevalence of overt DIC (80%) in drowning compares with 63% in our APL patients, to 33% reported for CA (22) and to 10–50% published for sepsis (23).

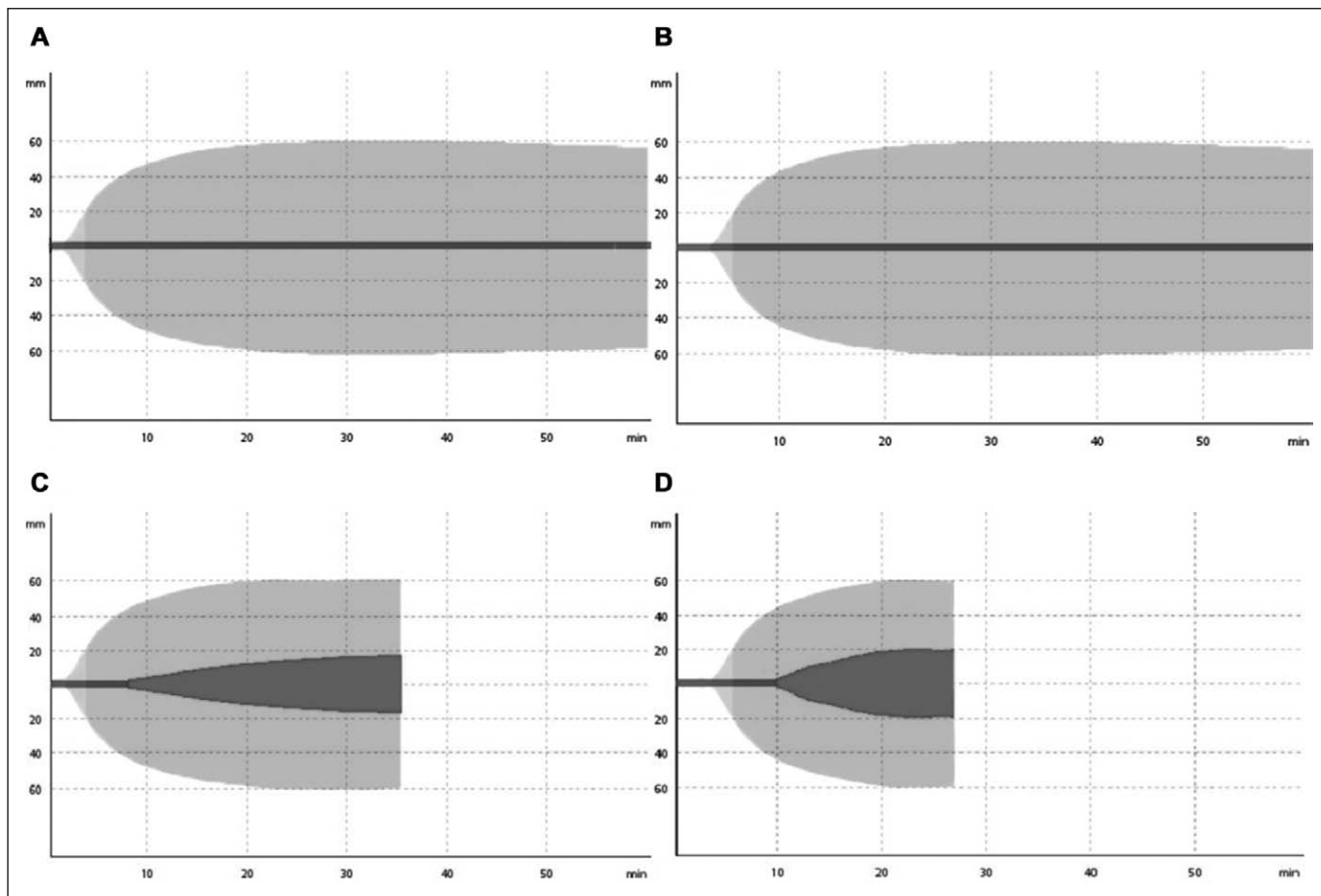
### Cause of Overt DIC Patterns in Drowning Patients

On the basis of our findings, we consider massive hyperfibrinolysis as main cause of overt DIC in drowning, reflected clinically by profuse bleeding, a complete absence of clotting in ROTEM analysis (and its reversal by antifibrinolytics), and high d-dimer as well as low fibrinogen levels accompanied by a marked APTT prolongation in coagulation studies. Hence, low fibrinogen and high d-dimer levels, which both well correlated with APTT, mainly contributed to the DIC score in drowning, even when compared with APL. Besides elevated d-dimer levels (which were still significantly lower than that of drowning victims), thrombocytopenia was the main contributor to DIC in APL (Fig. 2). APTT prolongation by hyperfibrinolysis in drowning is likely based on the high assay sensitivity to fibrinogen, explaining the good correlation between both parameters. In contrast, no correlation between APTT and fibrinogen was seen in our APL cohort although prolonged APTT is a known risk factor associated with hemorrhagic complications in APL (24). This may indicate a threshold effect requiring very low fibrinogen levels to affect the APTT assay.

Regarding the mechanism of hyperfibrinolysis, we consider severe ischemic hypoxia with ensuing release of tPA and possibly other fibrinolytics responsible: this is based on our findings that 1) plasma tPA levels of drowning patients were elevated 100-fold the normal limit, 2) the concomitant absent clotting signature in ROTEM was reversed by antifibrinolytics and reproduced by patient plasma in mixing studies, and 3) experimental forearm-ischemia increased tPA levels 31-fold.

This proposed mechanism corresponds to studies on the magnitude of fibrinolysis in traumatic coagulopathy reporting a significant correlation between systemic tPA levels and markers of hypoperfusion (25, 26). Hence, tranexamic acid together with fibrinogen substitution—both established therapeutics in trauma patients—may also be a rational, yet unproven, therapeutic approach for bleeding drowning victims.

Our *in vitro* experiments identified 10 ng/mL r-tPA as threshold to induce fibrinolysis in ROTEM analysis. Hence, tPA levels of drowning patients ( $23.5 \pm 5.6$  ng/mL) were sufficient to cause thrombelastometrically detectable hyperfibrinolysis. However, much higher amounts of r-tPA were necessary *in vitro* to completely abolish clotting (as seen in all drowning victims at admission). This discrepancy could indicate that we have missed the peak tPA release due its short half-life or that other fibrinolytic proteins contribute to hyperfibrinolysis in drowning patients.



**Figure 5.** Rotational thrombelastometric analysis traces of a drowning patient at admission. **A**, Tissue factor activates extrinsic hemostasis (EXTEM), **(B)** kaolin activates contact phase (INTEM), **C**, aprotinin abolishes lysis (APTEM), and **(D)** heparinase inhibits heparin effect (HEPTEM). EXTEM-clotting time (CT) and INTEM-CTs were greater than 5,400 s (maximum running time). Adding heparinase (HEPTEM) markedly shortened CT to  $598 \pm 121$  s, indicating autoheparinization. Adding aprotinin (APTEM) yielded similar results (CT shortening to  $544 \pm 87$  s), suggesting hyperfibrinolysis.

Accordingly, we could not induce hyperfibrinolysis by forearm-ischemia, despite a 31-fold increase of plasma tPA levels. However, the maximum tPA level reached by ischemia (6.8 ng/mL) was not sufficient to cause hyperfibrinolysis. This could be caused by loco-anatomical differences in tPA synthesis, storage, and release, which may preferentially occur in specific vascular beds (like the splanchnic vasculature) with high endothelial lytic capacity (27). Another explanation may be the greater degree of tissue hypoxia that occurs prior to blood flow cessation in drowning patients when compared with nondrowning CA. Although tissue hypoxia usually follows CA due to systemic hypoperfusion, asphyxia is the main causative event in drowning patients preceding CA. Drowning patients may, therefore, be considered as primarily global hypoxic patients with ensuing CA without immediate initiation of resuscitation and consequently long no-flow times. Hence, our arm-ischemia model resembles CA (i.e., hypoperfusion) rather than drowning (asphyxia with ensuing systemic hypoxia).

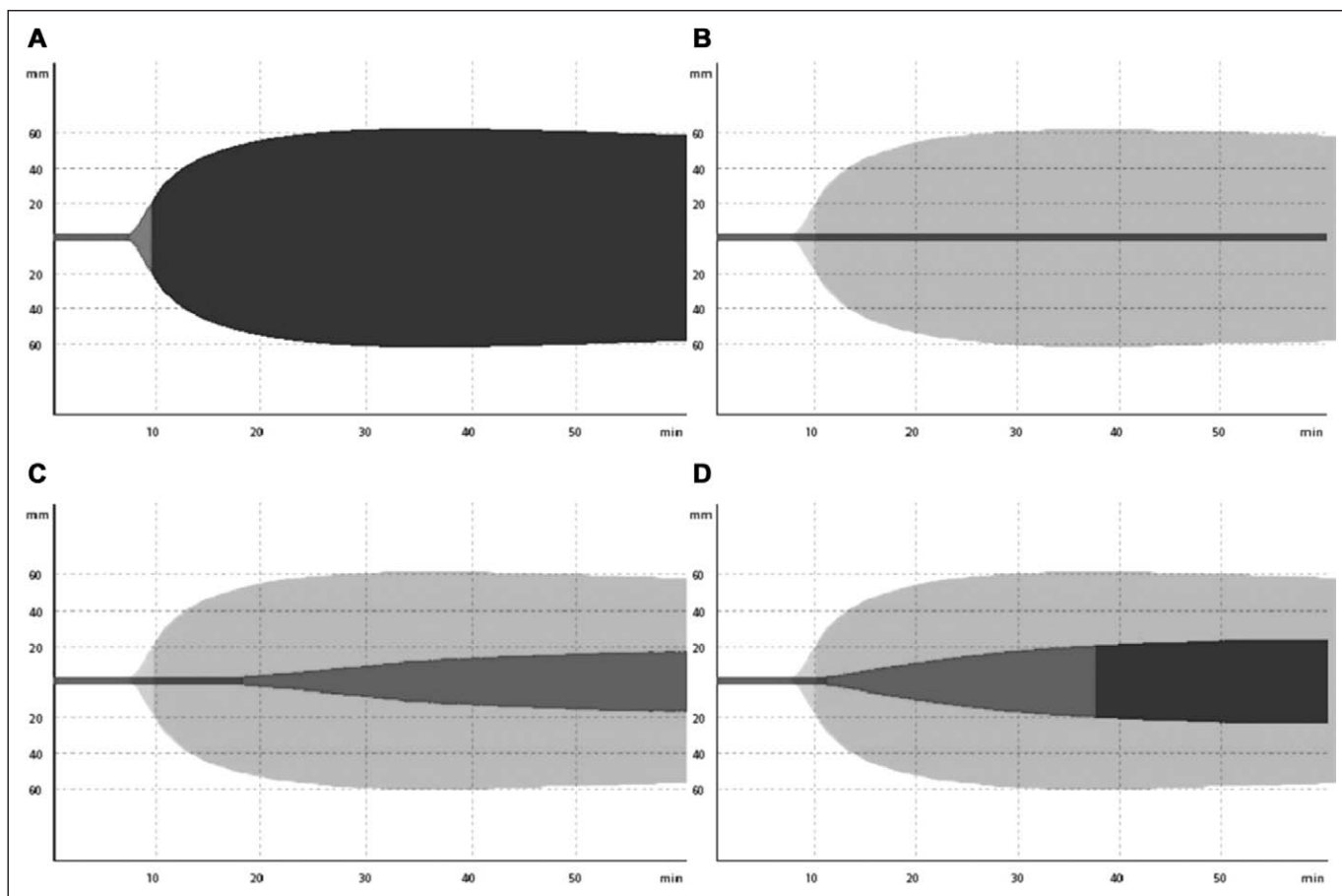
CPR times were significantly longer in drowning than in nondrowning CA patients, suggesting a possible relationship between CPR duration and DIC severity. However, no correlation was found between both parameters in drowning patients. The reliability of this finding may yet be limited because CPR duration in

drowning does not reflect the duration of asphyxia. Submersion time usually can only be estimated, depending on whether the submersion event was witnessed or not. Furthermore, in drowning, the exact time point when CA occurs is difficult to determine. Due to the low trustability on CPR time-data in drowning patients, we primarily chose the pH value ( $\text{pH} \leq 7.0$ ) as discriminating surrogate parameter of disease severity for subgroup analysis.

### Endogenous Heparinization

A further contributor to coagulopathy in drowning might be an endogenous heparin-like factor, as suggested by our findings that 1) exogenous r-tPA increased APTT only with a ceiling effect, 2) tPA levels detected in drowning patients were too low to completely abolish clotting in vitro, and 3) heparinase effectively shortened both the CT and the APTT in admission samples of drowning patients.

Endogenous heparinization following endothelial-glycocalyx disruption was recently described as contributing mechanism of traumatic coagulopathy (28). Contrary, neither syndecan-1 nor endothelial heparan sulfate levels were elevated in drowning patients, suggesting other/further heparinase-sensitive glycosaminoglycans responsible for drowning-related endogenous



**Figure 6.** Rotational thrombelastometric analysis trace of a reconstituted blood sample without and after the administration of antifibrinolytics. **A**, Nonactivated test using a recalcifying starting reagent trace of a whole-blood sample made up from two healthy volunteers with the same blood group. **B**, Mixing of thawed plasma from a drowning patient with blood cells from a healthy volunteer completely abolished clotting (clotting time [CT] > 5,400 s). **C**, This was markedly reversed by adding tranexamic acid (0.15 mg/mL, resulting in a normal CT of 1,045 s). **D**, CT was further shortened by adding both tranexamic acid and aprotinin (CT of 652 s). The low maximum clot firmness (23 mm) was caused by a low platelet count ( $51 \times 10^9/L$ ).

heparinization. Therefore, we tested tryptase level as surrogate parameter of mast cell-degranulation with ensuing heparin release as potential cause of endogenous anticoagulation; however, tryptase levels were normal (Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/B400>).

### Acidosis and Hypothermia

Acidosis impairs coagulation *in vitro* and *in vivo* (29–31). Accordingly, we found that whole-blood acidification mildly increased CT. Yet, even highest amounts of acid did not affect coagulation studies, despite a maximum increase in APTT to 68 seconds. Such a low pH value, however, was not detected in any patient. Furthermore, all patients with severe diabetic ketoacidosis had normal coagulation studies, suggesting that acidosis is not the sole factor of coagulation derangement. Whether the specific type of acidosis plays a role (lactic vs ketoacidosis) remains unknown.

Coagulation impairment by hypothermia has also been assessed in various studies with different results (32–34). However, despite its clinical relevance, an influence of hypothermia on coagulation studies in our drowning patients may be questionable because blood samples got rewarmed to 37°C before testing. The disparity between clinically evident

hypothermic coagulopathy and near-normal clotting studies is known and must be taken into account (35). The same applies to ROTEM analysis, which was done only after incubating samples at 37.0°C according to manufacturer's instructions. Dirkmann et al (29) found an inhibiting effect of hypothermia on fibrinolysis assessed by ROTEM. In agreement, we detected no impact of hypothermia on thrombelastometry in our forearm-ischemia model. Therefore, we considered hypothermia unlikely as mechanism of hyperfibrinolysis and ensuing coagulopathy by drowning. However, this issue has to be addressed in further studies.

### Limitations

In a land-locked country such as Austria, drowning accidents with consecutive hospital-admission are fortunately scarce. Thus, only six patients could be investigated prospectively within past years. However, the magnitude of effect sizes is large enough to provide highly significant differences when compared with normal values.

We investigated only freshwater accidents. Studies in other countries may be interesting to extend our findings to saltwater drowning.



Activated protein C (APC) could not be measured due to limited plasma samples. In traumatic coagulopathy, APC is implicated in the inhibition of plasminogen activator inhibitor-1 function (26), which may also be true for drowning patients. However, this supposed mechanism may be called into question, considering that even high concentrations of APC (60–80 ng/mL) did not decrease plasminogen activator inhibitor-1 activity in a previous study (20).

## CONCLUSIONS

Overt DIC occurs in the vast majority of drowning patients and is accompanied by clinically manifest bleeding. Ischemia-induced tPA release mechanistically contributes to the underlying hyperfibrinolysis and antifibrinolytics and heparinase partially reverse the abnormal clotting patterns. APTT prolongation may be a marker of the severity of hyperfibrinolysis.

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