Forgot calcium? Admission ionized-calcium in two civilian randomized controlled trials of prehospital plasma for traumatic hemorrhagic shock

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BACKGROUND: Randomized clinical trials (RCTs) support the use of prehospital plasma in traumatic hemorrhagic shock, especially in long transports. The citrate added to plasma binds with calcium, yet most prehospital trauma protocols have no guidelines for calcium replacement. We reviewed the experience of two recent prehospital plasma RCTs regarding admission ionized-calcium (i-Ca) blood levels and its impact on survival. We hypothesized that prehospital plasma is associated with hypocalcemia, which in turn is associated with lower survival.

METHODS: We studied patients enrolled in two institutions participating in prehospital plasma RCTs (control, standard of care; experimental, plasma), with i-Ca collected before calcium supplementation. Adults with traumatic hemorrhagic shock (systolic blood pressure ≤70 mm Hg or 71–90 mm Hg + heart rate ≥108 bpm) were eligible. We use generalized linear mixed models with random intercepts and Cox proportional hazards models with robust standard errors to account for clustered data by institution. Hypocalcemia was defined as i-Ca of 1.0 mmol/L or less.

RESULTS: Of 160 subjects (76% men), 48% received prehospital plasma (median age, 40 years [interquartile range, 28–53 years]) and 71% suffered blunt trauma (median Injury Severity Score [ISS], 22 [interquartile range, 17–34]). Prehospital plasma and control patients were similar regarding age, sex, ISS, blunt mechanism, and brain injury. Prehospital plasma recipients had significantly higher rates of hypocalcemia compared with controls (53% vs. 36%; adjusted relative risk, 1.48; 95% confidence interval [CI], 1.03–2.12; $p = 0.03$). Severe hypocalcemia was significantly associated with decreased survival (adjusted hazard ratio, 1.07; 95% CI, 1.02–1.13; $p = 0.01$) and massive transfusion (adjusted relative risk, 2.70; 95% CI, 1.13–6.46; $p = 0.03$), after adjustment for confounders (randomization group, age, ISS, and shock index).

CONCLUSION: Prehospital plasma in civilian trauma is associated with hypocalcemia, which in turn predicts lower survival and massive transfusion. These data underscore the need for explicit calcium supplementation guidelines in prehospital hemotherapy. (*J Trauma Acute Care Surg.* 2020;88: 588–596. Copyright © 2020 American Association for the Surgery of Trauma.)

LEVEL OF EVIDENCE: Therapeutic, level II.

KEY WORDS: Trauma-induced coagulopathy; coagulation; calcium; hemorrhage; transfusion.

Resuscitation techniques using blood components have progressed from crystalloid alone to balanced blood components and early plasma resuscitation in high-risk patients. These strategies were introduced for patients with liver injuries[1] and later expanded to all patients at risk of massive transfusion.[2] In the last 15 years, observational studies in military settings showed reduced mortality with high ratios of plasma to red blood cells (RBCs) during resuscitation.[3] Subsequently, observational civilian trauma studies reproduced these outcomes.[4] While a civilian randomized clinical trial (RCT) failed to confirm this benefit on 24-hour and 30-day mortality, it decreased hemorrhagic deaths at 24 hours.[5]

An evolving concept of plasma-first resuscitation was postulated,[6] and the Department of Defense funded two RCTs in civilian settings to test these effects. The Prehospital Plasma during Air Medical Transport in Trauma Patients at Risk of Hemorrhage (PAMPer) trial[7] demonstrated a significant mortality reduction in air-transported patients, who received prehospital plasma compared with normal saline. Conversely, the Control of Major Bleeding After Trauma (COMBAT) trial[8] did not demonstrate a survival advantage with early plasma in a short transport, ground-ambulance system. Differences in the proportion of blunt versus penetrating mechanism (COMBAT had more penetrating injuries than PAMPer)[9] and transport time (COMBAT had shorter transport time than PAMPer)[10] were invoked to explain the disparity in the outcomes of these two RCTs.

Hypocalcemia could be a factor contributing to dampen the benefit of prehospital citrated plasma, as studies in prehospital and hospital settings have detected it in association with transfusions.[11–13] The discovery that citrate rendered whole blood incoagulable was a major advance in transfusion medicine.[14] Although the effect of citrate on coagulation via binding calcium was known since the 1800s, it was only in 1915 that several physicians around the globe used sodium citrate as an anticoagulant to transform the transfusion procedure from direct to indirect.[14,15] During World War I, Oswald Robertson,[16] an American Army officer, considered the developer of the first blood bank, published a remarkable paper describing transfusions of stored red cells given near the front line in France. However, the residual iatrogenic effect of reduced circulating calcium due to binding with citrate in patients following transfusion, which had been appreciated for over 100 years, fueled caution.[17] Adams et al.[18] in 1944 reported that *citrate intoxication* driving lethal hypocalcemia in animals was found to occur after greater than 60% blood loss was replaced with citrated whole blood, but was rapidly reversible with calcium gluconate. These authors warned that administration of plasma was even more concerning, as the amount of citrate in plasma was greater than that in citrated blood. However, because of the perceived rarity of severe hypocalcemia from citrated blood product transfusion, its clinical significance was questioned[19]; one study even warned against "the potential dangers of intravenous calcium salt therapy." Guidelines in the 1960s,[20] however, cautioned for the risks of hypocalcemia associated with citrate intoxication due to rapid

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TABLE 1. Patient Characteristics and Outcomes Stratified by Study Group

	Control Group (n = 84, 52.5%), n (%) or Median (IQR)	Experimental Group (n = 76, 47.5%), n (%) or Median (IQR)	*p*
Trial			
COMBAT	33 (39.3%)	30 (39.5%)	1.00
PAMPer	51 (60.7%)	46 (60.5%)	
Age, y	39.5 (26–52)	41 (29–54)	0.72
Female sex	18 (21.4%)	20 (26.3%)	0.58
ISS	23 (17–33)	22 (15–35)	0.56
Field HR, bpm	115 (108–128)	119 (109–127)	0.58
Field SBP, mmHg	73 (61–83)	72 (63–81)	0.83
Field shock index	1.53 (1.37–1.87)	1.60 (1.38–1.85)	0.49
Field Glasgow Coma Scale	12 (3–15)	14 (3–15)	0.32
Minutes to hospital arrival	35 (20–54)	39 (21–55)	0.62
Blunt mechanism	64 (76.2%)	50 (65.8%)	0.16
TBI (AIS head, >2)	29 (34.5%)	24 (31.6%)	0.74
Calcium			
Minutes to first i-CA	41 (9–110)	47 (22–110)	0.63
First i-Ca, mmol/L	1.03 (0.97–1.09)	0.99 (0.94–1.06)	0.02
Hypocalcemia (i-Ca, ≤1.0 mmol/L)	30 (35.7%)	40 (52.6%)	0.03
Ca supplementation received	58 (69.1%)	45 (59.2%)	0.11
Minutes to Ca supplementation	126 (61–469)	99 (43–228)	
Physiology/coagulation			
Admission pH	7.24 (7.15–7.33)	7.25 (7.18–7.32)	0.66
Admission hemoglobin, g/dL	12.7 (11.2–14.1)	12.3 (10.3–14.3)	0.29
Admission prothrombin time/INR	1.26 (1.10–1.50)	1.2 (1.1–1.4)	0.39
R-TEG R, s	0.8 (0.7–1.0)	0.8 (0.8–0.9)	0.72
R-TEG MA, mm	56.5 (49.0–60.6)	56.5 (51.9–62.9)	0.40
R-TEG angle, degrees	69.4 (61.1–73.1)	70.5 (64.5–75.3)	0.42
R-TEG LY30, %	0.7 (0.1–2.1)	0.2 (0–1.7)	0.13
Transfusions/fluids/tranexamic acid			
RBC units/24 h	3 (0–8)	2 (0–8)	0.96
Prehospital RBC received (PAMPer only)	6 (15.8%)	4 (12.1%)	0.74
Massive transfusion (>10 RBC units or death/24 h)	15 (17.9%)	16 (21.1%)	0.69
Plasma units/24 h	0 (0–4)	2 (2–7)	<0.001*
Platelet units/24 h	0 (0–1)	0 (0–1)	0.95
Cryoprecipitate units/24 h	0 (0–0)	0 (0–0)	0.48
Field Crystalloids, mL	350 (150–800)	250 (0–750)	0.17
Tranexamic acid administered	2 (2.4%)	0	0.50
Outcomes			
ICU days	5 (2–14)	7 (2–14)	0.52
Mortality	15 (17.9%)	9 (11.8%)	0.38

*By design.

R-TEG, rapid thrombelastogram; MA, maximum amplitude; ICU, intensive care unit; INR, international normalized ratio; TBI, traumatic brain injury; Ca, calcium; IQR, interquartile range; AIS, Abbreviated Injury Scale; RBC, red blood cells; ISS, injury severity score; HR, heart rate; SBP, systolic blood pressure.

administration of blood and recommended 10 mL of 10% calcium gluconate be given for each liter of blood transfused.

Recent data suggest that severe hypocalcemia is common in patients undergoing massive transfusion,[11] and severe hypocalcemia has been associated with an increased risk of death.[21] A recent military analysis found that hypocalcemia was as common as 70% in patients treated with prehospital blood products.[13] Nevertheless, the 2018 Advanced Trauma Life Support guidelines[22] do not recommend calcium supplementation for most transfusions.

The two prehospital RCTs (COMBAT and PAMPer)[7,8] provided an opportune setting to address the effects of early citrated plasma resuscitation on calcium homeostasis. We hypothesize that patients who received prehospital plasma compared with crystalloid resuscitation would be at a higher risk of hypocalcemia upon presentation to the hospital, and those patients with hypocalcemia would be at increased risk of mortality and massive transfusion.

PATIENTS AND METHODS

We included adults with traumatic hemorrhagic shock (systolic blood pressure [SBP] ≤70 mm Hg or 71–90 mm Hg + heart

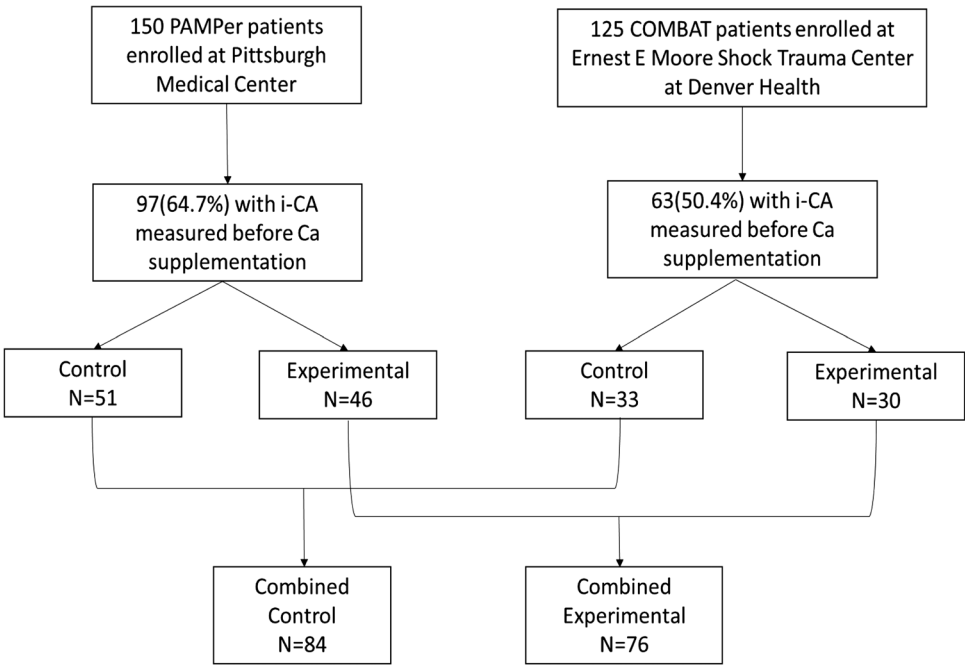

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram.

rate [HR] ≥108 bpm) enrolled in two institutions participating in two prehospital plasma RCTs: (1) University of Pittsburgh Medical Center, one of the trauma centers participating in the PAMPer trial,[7] and (2) Ernest E. Moore Shock Trauma Center at Denver Health in Denver, Colorado, the single center in the COMBAT trial.[8] We were unable to obtain ionized calcium (i-Ca) levels from the other facilities participating in PAMPer. Both trials were approved by the Food and Drug Administration, the Human Research Protection Office of the Department of Defense, and the institutional review boards at the participating sites. Both trials were pragmatic and designed simultaneously with similar methods to be subsequently harmonized.[7,8] In brief, adult patients (COMBAT, age >18 years; PAMPer, 18–90 years) with hemorrhagic shock (SBP ≤ 70 mm Hg or 71–90 mm Hg with HR ≥108 bpm) were randomized in the field to receive either 2 U of universal donor (AB) thawed plasma (experimental) or standard of care (control—COMBAT, normal saline; PAMPer, normal saline with or without RBCs if required). Exclusion criteria were prisoner status, known pregnancy, isolated penetrating injury to the head, asystole or cardiopulmonary resuscitation before randomization, known objection to blood products, opt-out bracelets or necklaces, or family objection to the patient's enrolment. PAMPer also excluded documented cervical cord injury. The major difference between trials was the mode of transport: PAMPer was limited to patients transported by air, using nurse-paramedic teams over relatively long distances, compared with COMBAT patients, transported by Denver Health affiliated ambulances, using paramedics over short distances in Denver City and County.

For this study, we selected patients with blunt or penetrating injuries, for whom i-Ca was collected before calcium supplementation. Although i-Ca measurement was not part of the studies' protocols, it was often obtained as part of the resuscitation protocol of both institutions. The i-Ca was measured in arterial blood gas samples, thus reflecting actual free calcium in vivo concentrations regardless of altered pH and plasma proteins. Notably, the arterial pH of these patients upon admission was similar in the two study groups (Table 1). Hypocalcemia was defined as in previous studies as i-Ca of 1.0 mmol/L or less.[11] Hypercalcemia was defined as i-Ca of greater than 1.25 mmol/L.[21] Traumatic brain injury was defined as Abbreviated Injury Scale score for head/neck greater than 2. Injury severity was assessed by the Injury Severity Score (ISS). Massive transfusion was defined as greater than 10 RBC units or death within 24 hours postinjury.

Statistical Analysis

We expressed numerical variables as median and interquartile range and categorical variables as number and percent. We performed univariate comparisons with χ^2 or Fisher exact tests for categorical variables and with *t* test or Mann-Whitney *U* test for numerical variables. We used generalized linear mixed models (GeLMs) with random intercepts and Cox proportional hazards models with robust standard errors to account for clustered data by institution/trial. For analysis of survival and massive transfusion, variables fitting the definition of a confounder (i.e., associated with both the outcome and the exposure) and univariately associated with the outcome at $p < 0.25$ were included in the models. Violations of the proportionality assumption were checked and, when present, remedied by entering an interaction of the offending variable with time. Kaplan-Meier curves illustrated the survival by i-Ca strata. All tests were two-tailed with significance established at $p < 0.05$. The analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Of 150 PAMPer subjects admitted to the University of Pittsburgh Medical Center, 97 (64.7%) had i-CA measured

TABLE 2. Patient Characteristics Stratified by Normocalcemia (i-Ca, >1.0 mmol/L) and Hypocalcemia (i-Ca, ≤1.0 mmol/L)

	Normocalcemia, i-Ca >1.0 mmol/L (n = 90), n (%) or Median (IQR)	Hypocalcemia, i-Ca ≤1.0 mmol/L (n = 70), n (%) or Median (IQR)	*p*
Randomized to plasma group	36 (40.0%)	40 (57.1%)	0.03
Trial = COMBAT	32 (35.5%)	31 (44.3%)	0.33
Trial = PAMPer	58 (64.4%)	39 (55.7%)	
Age, y	43 (26–58)	36 (28–51)	0.17
Female sex	22 (24.4%)	16 (22.9%)	0.81
ISS	22 (13–20)	27 (17–34)	0.05
Field SBP, mmHg	79 (68–86)	70	<0.001
Field HR, bpm	112 (108–122)	120	0.04
Field shock index	1.44 (1.33–1.63)	1.72	<0.001
Minutes to hospital	39 (22–54)	34 (20–55)	0.42
Blunt trauma	67 (74.4%)	47 (67.1%)	0.31
TBI (AIS head/neck, >2)	28 (31.1%)	25 (35.7%)	0.54
Calcium			
i-Ca (mmol/L)	1.08 (1.04–1.11)	0.95 (0.86–0.97)	<0.0001
Minutes to i-Ca measurement	33 (18–218)	37 (20–196)	0.16
Coagulation			
Prothrombin time/INR	1.20 (1.10–1.40)	1.31 (1.16–1.50)	0.11
R-TEG ACT, s	121 (105–136)	121 (105–128)	0.43
R-TEG angle, degrees	70.8 (65.3–75.8)	67 (60.6–72.2)	0.02
R-TEG MA, mm	57.5 (53.8–64.4)	54 (38.7–59.9)	0.005
R-TEG LY30, %	0.70 (0–2.0)	0.5 (0–2.0)	0.69
Hyperfibrinolysis (LY30 >7.6%)	3 (6.3%)	2 (5.0%)	0.82
Physiologic lysis (LY30 0.6–7.6%)	23 (47.9%)	17 (42.5%)	
Lysis shutdown (LY30 <0.6%)	22 (45.8%)	21 (52.5%)	
Transfusions/fluids/tranexamic acid			
RBC units/24 h	1 (0–5)	5 (2–10)	0.0002
Massive transfusion (>10 RBC units or death/24 h)	11 (12.2%)	20 (28.6%)	0.009
Plasma units/24 h	2 (0–4)	2 (1–7)	0.007
Platelet units/24 h	0 (0–0)	0 (0–1)	0.30
Cryoprecipitate units/24 h	0 (0–0)	0 (0–0)	0.0003
Field Crystalloids, mL	250 (100–600)	400 (0–800)	0.43
Tranexamic acid administered	1 (1.1%)	1 (1.4%)	1.00
Outcomes			
ICU days	6 (2.0–11.0)	6 (2–17)	0.59
Mortality	11 (12.2%)	13 (18.6%)	0.26

R-TEG, rapid thrombelastogram; MA, maximum amplitude; LY30, % fibrinolysis 30 minutes after MA; ICU, intensive care unit; INR, international normalized ratio; TBI, traumatic brain injury; IQR, interquartile range; AIS, Abbreviated Injury Scale; RBC, red blood cells; ISS, injury severity score; HR, heart rate; SBP, systolic blood pressure.

before calcium supplementation, while in COMBAT, 63 (50.4%) of 125 subjects had this measurement (Fig. 1). This resulted in a study sample of 160 subjects, of whom 48% received prehospital plasma. Table 1 shows the characteristics of these patients, stratified by experimental (prehospital plasma) and control (prehospital normal saline) groups. The groups were well balanced regarding baseline characteristics. Of note, the groups had similar median time to first i-Ca measurement before calcium supplementation: 41 minutes (9–110 minutes) in the control group and 47 minutes (22–110 minutes) in the prehospital plasma group ($p = 0.63$). The differences in mortality and massive transfusions between the experimental and control groups were not statistically significant.

Overall, 44% had hypocalcemia, but the experimental group had a higher incidence of hypocalcemia than the control group (35.7% vs. 52.6%, $p = 0.03$), which remained

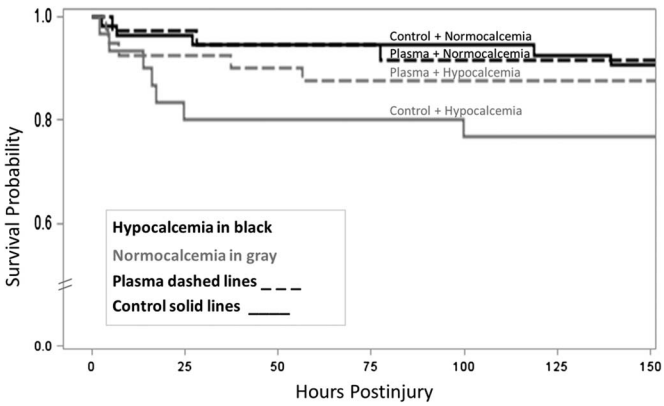

Figure 2. Kaplan-Meier survival curves by study group (prehospital plasma and control) and hypocalcemia (i-Ca, ≤1.0 mmol/L).

TABLE 3. Cox Proportional Hazards Model Testing the Effect of Hypocalcemia on Survival Adjusted for Age, ISS, and Shock Index

Variable	Hazard Ratio	95% Hazard Ratio Confidence Limits		*p*
Hypocalcemia	1.07	1.02	1.13	0.01
Experimental vs. control group	0.92	0.75	1.12	0.40
Age (10 y)	1.02	1.02	1.03	<0.0001
ISS (10 points)	1.04	1.01	1.07	0.004
Shock index (1.00 U)	1.02	1.01	1.03	0.002

significant after accounting for clustered data by institution/trial (GeLM, $p = 0.03$; adjusted relative risk, 1.48; 95% confidence interval [CI], 1.03–2.12). Only two individuals had mild hypercalcemia (1.42 and 1.55 mmol/L, one in each study site, none received prehospital plasma, and both survived).

Table 2 depicts the characteristics of patients with and without hypocalcemia. Mortality in the hypocalcemia group was 18.6% compared with 12.2% in the normocalcemia group. Massive transfusion was required in 28.6% of patients with hypocalcemia and in 12.2% of patients with normocalcemia. Hypocalcemia was associated with significantly lower angle and maximum amplitude.

Kaplan-Meyer curves (Fig. 2) showed that the control group with hypocalcemia had the lowest survival, followed by the experimental group with hypocalcemia. Confounder candidates for the association of hypocalcemia with death and with massive transfusion were as follows: age, ISS, SBP, HR, and shock index upon admission as well as randomization group (Table 2). Because SBP and HR are components of the shock index (thus highly correlated), we entered shock index in the Cox proportional hazards model as a confounder of the association of hypocalcemia with survival. Hypocalcemia was independently associated with decreased survival (Table 3). A quadratic term testing a U-shaped association of i-Ca levels with survival was tested; because it was not significant, it was dropped from the model. In addition, using the GeLM to adjust for the above confounders and account for clustered data by institution/trial, hypocalcemia was significantly associated with massive transfusion (adjusted relative risk, 2.70; 95% CI, 1.13–6.46; $p = 0.03$).

DISCUSSION

Combining two RCTs that tested plasma-first resuscitation in the prehospital setting identified a significant risk of hypocalcemia after administration of citrated blood products. Hypocalcemia was present in roughly one third of patients who did not receive plasma, compared with over half in patients who received plasma. Hypocalcemia was an independent predictor of mortality and of massive transfusion after adjusting for confounders (age, injury severity, and shock index). While hypocalcemia appeared to dampen the survival benefit of prehospital plasma (Fig. 2), the current study was not designed nor powered to assess the effect of the prehospital plasma on survival or massive transfusion.

Optimal coagulation requires calcium as well as close-to-normal acid-base balance, hematocrit, and temperature; if these essential elements are missing, hemostatic therapy may be in vain as stable clotting cannot occur.[23] Calcium plays an essential role in the formation and stabilization of fibrin polymerization sites, and consequently, it has an impact on all platelet-dependent functions. Nevertheless, laboratorial coagulation tests may mask the negative impact of hypocalcemia on coagulation, as blood samples are recalcified before being assayed. In addition, calcium is essential in myocardial and vascular smooth muscle contraction.

Hypocalcemia's association with blood product use in these two RCTs confirms the recent military and civilian experience.[11–13,21] Our study, however, advances this knowledge by examining the specific role of prehospital plasma and demonstrating that even a relatively small volume of a blood component may decrease i-Ca levels.

The cause of hypocalcemia following hemorrhagic shock and injury remains unclear, yet there is an alarming paucity of recently published primary data addressing causes and interventions.[24] Indeed, in the 34th William Fitts Jr Oration, presented at the 67th Annual Meeting of the American Association for the Surgery of Trauma in 2008, Charles E. Lucas.[25] summarized his group's decades-long work on this topic and alerted for the little scientific attention given to calcium. Moreover, gaps in knowledge about the citrate content in blood products and its impact on i-Ca seem to exist. A recent survey of a large, urban, academic level I trauma center in the northeast United States revealed that the majority of the respondents erroneously believed that RBCs were the blood component with the largest amount of citrate and ignored that plasma and platelets contained 60% of the total citrate.[26,27]

Mechanisms other than citrated blood products are involved in the development of hypocalcemia. Indeed, Vivien et al.[28] compared observed i-Ca and expected i-Ca (corrected for hemodilution, pH, lactate and colloid binding of calcium) upon admission in trauma patients before receiving any blood component and noted a significant difference between them in severely injured patients, presumably because of shock and/or ischemia-reperfusion. Another potential causal mechanism for hypocalcemia could be low hepatic clearance of citrate due to defective hepatic perfusion caused by the hemorrhagic shock.[29] Lucas et al.[30] in the early 1980s demonstrated that low total calcium and i-CA in 41 injured patients who required blood transfusions were associated with shock time and hypoalbuminemia, as well as blood products and crystalloid administration during resuscitation. Further investigation in animals by this group suggested a direct association between shock and hypocalcemia.[31]

Our findings regarding the association of hypocalcemia with death and the need for massive transfusion confirm the observations of other investigators.[32] Our study was also consistent with the previous association of increased mortality in patients with hypocalcemia who were critically ill because of reasons other than trauma[33] and with a large retrospective study of 15,000 critically ill patients, in which hypocalcemia remained a predictor of mortality.[34]

Limitations of this study include the lack of i-Ca measurements in all patients enrolled in the RCTs, although a very good balance in baseline risk factors was retained in the subgroup with these measurements, strongly suggesting that selection bias was not at play. This preserved the internal validity of the

assessment of the association between prehospital plasma with hypocalcemia. In contrast, the modifying effects of preexisting disease severity may have confounded the impact of hypocalcemia on survival. It is important to note that mode of transport (air transport in PAMPer and ground in COMBAT) is also a surrogate for different levels of care (a critical care team nurse + medic vs. a single medic in the back of the ambulance), potentially introducing some survivor bias (patients have to survive long enough for helicopter to arrive on-site) and selection bias (air transport is only activated if patient care warrants resources unavailable locally and for more severely injured patients). These biases can potentially limit the generalizability of our results (i.e., less severely injured patients may have lower incidence of hypocalcemia).

We did not conduct analyses on the impact of calcium replacement on survival, as these would be severely flawed because of survivor bias (patients must be alive to receive it), intervention bias (patients with more severe injuries and/or in-extremis are more likely to receive it), and incomplete risk adjustment due to small sample size. Of note, although calcium replacement is usually part of in-hospital massive transfusion protocols, this is not the case in prehospital plasma-first resuscitation, or in patients who require less blood products than those for whom massive transfusion protocols are activated. Collectively, these deficiencies underscore the need for large prospective trials testing the signal detected in these solid analyses of the association of citrated-plasma resuscitation with hypocalcemia.

In summary, trauma patients resuscitated with prehospital plasma often present to the hospital with hypocalcemia, which place them at increased risk of mortality. Citrate in the plasma contributes to hypocalcemia, but other causes of low i-Ca remain unclear because some patients who did not receive plasma also had hypocalcemia. Thus, further research into the mechanisms of postinjury hypocalcemia and associated mortality is needed. A randomized controlled trial will be required to provide definitive answers regarding the optimal therapeutic interventions. Nevertheless, while such evidence is unavailable, prehospital administration of calcium is a simple, relatively innocuous approach to attenuate injury- and resuscitation-induced hypocalcemia, particularly in patients who received blood products. The recently published European guidelines[35] favored 10% calcium chloride (270 mg of elemental calcium/10 mL) over 10% calcium gluconate (90 mg of elemental calcium/10 mL), especially in the presence of abnormal liver function, because decreased citrate metabolism results in slower release of i-Ca. It is important to note that both calcium salts should be given slowly to avoid cardiac effects, and that extravasation into soft tissue can cause severe lesions.[36] Because of the low risk of iatrogenic effects of calcium gluconate, we advocate that 1 g (10 mL) be given for every 1 to 2 U of blood products (plasma inclusive) and that admission i-Ca levels be monitored early and often in patients with hemorrhagic shock.

AUTHORSHIP

All authors (H.B.M., M.T.T., E.E.M., J.L.S., M.J.C., M.P.C., A.E.P., F.X.G., J.B.B., M.N., B.Z., and A.S.) contributed in the design and interpretation of data. A.S. and H.B.M. also contributed in the analysis. J.L.S., A.S., and M.T.T. also contributed in the data acquisition. All authors were actively involved in the drafting and critical revision of the article and provided final approval of the version to be published.

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DISCLOSURE

For all authors, no conflicts are declared.
Contents are the authors' sole responsibility and do not necessarily represent official NIH or Department of Defense views.

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DISCUSSION

JEREMY W. CANNON, M.D. (Philadelphia, Pennsylvania): Dr. Mohr, Dr. Fildes, members and guests, first, I'd like to say thank you for the opportunity to discuss this important paper.

What we have seen here is a great example of servant leadership with Dr. Moore taking one of the team. I guess it shows that the "thrill" of being on the hot-seat never fades.

So with that, what we have just been treated to is a very sophisticated trial design that was intended to be harmonized from the start. You saw the inclusion criteria were synchronous. And, as a result, the analysis was able to be completed with these data sets combined. And this group has addressed a very pragmatic question.

Moore and colleagues explored whether there was an iatrogenic component to the hypocalcemia in the COMBAT and PAMPER studies and whether this hypocalcemia may have actually blunted some of the mortality benefit of pre-hospital plasma resuscitation.

So without recapitulating the results that you have heard so nicely presented, suffice it to say that mitigating that citrate toxicity that was a concern for so many years is actually a very important aspect of hemostatic resuscitation in these bleeding trauma patients. Despite the limitations of this post-hoc subset analysis I do believe that the common-sense approach that you have recommended of early empiric calcium dosing followed by frequent calcium laboratory assessments should be actually a quality indicator in both civilian and military trauma resuscitations.

So, other secondary outcomes were rare and underpowered to measure, such as maternal or fetal mortality, but these indeed merit future consideration on a larger scale. So with that, I have a few questions for the authors.

With that said I do have a couple of questions. First, regarding the study population, why did you exclude patients from those eight other centers in the PAMPER study? Of course if you had included those patients we would have a much larger sample size.

Second, regarding the pre-hospital packed red blood cells, you covered this well in your presentation but I am curious, did you look at the effects of citrate in the non-plasma patients? Was there an effect of from packed red blood cells on the hypocalcemia? Those patients actually received a much larger citrate dose than the plasma only patients which should be accounted for in some way in your analysis.

Third, regarding calcium trends. So calcium can fluctuate pretty dramatically through the course of a resuscitation. In a study we conducted at the University of Pennsylvania we found that it flew all over the place. So rather than just looking at the initial calcium level, did you also assess calcium trends through the resuscitation?

ZSOLT J. BALOGH, M.D. Ph.D. (Newcastle, Austria): Great study, Gene. I just would like to ask if you looked at the calcium concentrations as a continuous variable? And was there any cut-off point to tell apart from one when the better outcome starts to happen? Thank you very much.

And then what about that other pesky divalent cation, magnesium? As you know, magnesium tends to track with calcium, and very low levels can result in a malignant arrhythmia.

And then, finally, were you able to calculate a dose-response curve for citrate reversal? And on this topic, I was intrigued by your recommendation for calcium gluconate. I'm not sure I'm convinced that peripheral vein toxicity is compelling enough to steer away from calcium chloride which is a very familiar medication for pre-hospital personnel. In addition, as you showed, the elemental calcium in the gluconate formulation is about a third of calcium chloride and it's in three times the

volume. So I'm wondering if calcium gluconate may be a little bit unfamiliar to pre-hospital personnel and the potency may not be quite what we're looking for. If you could please further explain this recommendation, that would be great.

Thank you to the AAST for the privilege of the podium, and I look forward to the discussion.

AJAI K. MALHOTRA, M.D., M.Sc., M.B.B.A (Burlington, Vermont): Really enjoyed that study. But some patients developed hypocalcemia and some did not so is it possible that the hypocalcemia is a marker of say more severe injury or something so just giving calcium will not necessarily change the outcomes?

JAY J. DOUCET, M.D. (San Diego, California): Just a comment and a question. First a comment. The first use of citrate in blood transfusion in World War I was actually by the Canadian Army Medical Corps and Dr. Edward Archibald who didn't actually get much credit for it but actually published in 1916, a year before the U.S. actually entered the war.

So citrate was already in use but Oswald Robinson certainly popularized it because the Canadians had trouble convincing the British it was a good idea.

The other question I have is are these all arterial samples? Was there any venous sampling of blood? I ask because sometimes there are differences in how that blood is sampled such as toruniquets and in the additives in the syringe.

ROBERT A. COONEY, M.D. (Syracuse, New York): Gene, I was wondering if you came up with any evidence that calcium supplementation improves outcomes in severe injury or critical illness because I've looked at this in the past and although there is a clear association with hypocalcemia and worse outcomes I could never find any data that supported supplementing it would improve outcomes.

ERNEST E. MOORE, M.D. (Denver, Colorado): Thank you very much for your interest in this work, I will start with Dr. Cannon.

The eight other centers participating in PAMPER did not have consistent availability in documentation when calcium was administered because this was not part of the harmonization of the study. But certainly we encourage others to examine their multi-center trials to provide a larger population.

We agree the issue of the red cell contamination is important. Fortunately, the two groups, the crystalloid and the plasma, received equal amount of red cells.

Interestingly, there is more citrate in plasma and platelets than there is in red cells. We're often inclined to give calcium with red cells but forget that actually 60 percent of citrate is in these other components.

In terms of following the trends of calcium, as you observed at the University of Pennsylvania, we similarly were concerned there are just too many confounders to evaluate a subsequent calcium levels in these patients, particularly as many are undergoing a massive transfusion and receiving products and variable doses of calcium throughout.

The issue of the other ions that are also bound by citrate is interesting, magnesium being perhaps the most concerning. But the binding of magnesium with citrate is actually of relatively low affinity compared to, for example, EDTA.

Furthermore, it's interesting that citrate biologically is critical to transport manganese into cells. So citrate actually has a protective physiologic effect.

Regarding the dose response we are unable to suggest anything beyond what is reported in the literature, which is confusing. The best we can come up with is what we have recommended.

The issue of venous injury with calcium chloride versus calcium gluconate is controversial, but many guidelines suggest that the gluconate should be preferred over the chloride when dealing with peripheral access only.

Zsolt, we did assess ionized calcium concentration as a continuous variable; its adjusted hazard ratio showed that the ionized calcium level was significantly associated with death after adjustment for randomization group, ISS and field shock index ,and specifically the higher the ionized calcium level the lower likelihood of death). The Youden Index of ionized calcium was 1.0mmol/L, confirming the cutoff described in other studies of hypocalcemia in trauma patients requiring massive transfusion. We refrained from reporting the derivation of the Youden Index in the manuscript because the sample is not large enough for making this inference.

Dr. Malhotra, you have asked the important question about whether calcium makes a difference. I In our study we found it improved survival.

Clearly, stop the bleeding is more critical but calcium contributes to adverse outcomes; and, therefore, we do stand by our position that calcium should be supplemented in patients receiving rapid blood transfusion.

Dr. Doucet, I apologize for my oversight in reading the literature and certainly don't want to offend our Canadian colleagues.

Actually, when you look at the citrate story, it's interesting that citrate was first recognized to bind calcium and prevent coagulation of blood in the 1950s by Germans so there is a long history to this. I am sorry but we do not know whether samples were arterial or venous. Finally, Dr. Cooney, I agree we need to be careful how we translate this. Again, we found improvement in survival in our study I but there was a randomized trial in the intensive care unit showing that calcium supplementation was associated with adverse outcome even within normal ranges. But again, our study addresses acute resuscitation, not the later phase in the ICU. The ICU setting is a complex issue. In one of Dr. Lucas' presentations, in fact the Fitt's Oration, he suggested that in the ICU, particularly when compounded by renal failure, we have a greater risk of developing hypercalcemia and subsequent bradycardia and other cardiac events. So you are absolutely right, we need to be careful to view this in the context in which we apply it.

On behalf of our team, we thank you for your very astute questions.