Early & Empiric High-Dose Cryoprecipitate for Hemorrhage after Traumatic Injury: A Randomized Clinical Trial

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KEY POINTS

Question: Does transfusion of early and empiric, high dose cryoprecipitate in addition to standard care improve survival in bleeding trauma patients that require activation of a major hemorrhage protocol (MHP)?

Findings: In this multicenter, international, randomized clinical trial of 1604 trauma patients comparing standard care versus usual care, plus three early pools of cryoprecipitate there was no difference in all-cause 28-day mortality (26.1% vs 25.3%). There was no difference in safety outcomes, transfusion requirements or incidence of thrombotic events across study groups.

Meaning: The addition of early and empiric, high dose cryoprecipitate to usual care did not improve clinical outcomes in bleeding trauma patients.

STRUCTURED ABSTRACT

Importance

Critical bleeding is associated with a high mortality in trauma patients. Hemorrhage is exacerbated by a complex derangement of coagulation, including an acute fibrinogen deficiency. Management is fibrinogen replacement, with cryoprecipitate transfusions or fibrinogen concentrate, usually administered relatively late during hemorrhage.

Objective

To assess whether survival could be improved by administering an early and empiric, high dose of cryoprecipitate to all bleeding trauma patients that required activation of a major hemorrhage protocol.

Design, Setting & Participants

CRYOSTAT-2 was an interventional, randomized, open-label, parallel-group controlled, international, multicenter study. Patients were enrolled at 26 UK and US Major Trauma Centers from August 2017 to November 2021. Eligible patients were injured adults requiring activation of the hospital's major hemorrhage protocol with evidence of active hemorrhage, a systolic blood pressure <90 mmHg at any time and receiving at least one unit of a blood component transfusion.

Intervention

Patients were randomly assigned (1:1) to standard care (STD) which was the local MHP (reviewed for guideline adherence), or intervention (CRYO), in which three pools of cryoprecipitate (6g fibrinogen equivalent) were to be administered in addition to standard care, within 90 minutes of randomization and three hours of injury.

Main Outcomes and Measures

Primary outcome was all-cause mortality at 28 days in the intention-to-treat population (ITT).

Results

Among 1604 eligible patients, 799 were randomized to the CRYO and 805 to the STD groups. Missing primary outcome data occurred in 73 patients (principally due to withdrawal of consent), and 1531 (95%) were the primary analysis population. Median age 39 [IQR 26-55] years, male gender 79%, median injury severity score 29 [IQR 18-43], 36% had penetrating injury and 33% had systolic blood pressure <90 mmHg at hospital arrival. All-cause 28-day mortality in the ITT analysis was 26.1% (STD) vs 25.3% (CRYO) (OR 0.96 (95% CI 0.75-1.23), p=0.74). There was no difference in safety outcomes or incidence of thrombotic events, STD vs CRYO: 12.9% vs 12.7%.

Conclusions and Relevance

Among bleeding trauma patients who required activation of a major hemorrhage protocol, the addition of early and empiric, high-dose cryoprecipitate to standard care did not improve all cause 28-day mortality.

Trial Registration

ISRCTN (14998314) and ClinicalTrials.gov (NCT04704869).

INTRODUCTION

Hemorrhage contributes to over half of the annual 4.4 million trauma deaths worldwide, and one in four severely injured patients with major blood loss will die. Trauma causes a multifactorial clotting disorder which exacerbates bleeding and confounds surgical and/or resuscitative attempts at hemostasis[1].

Fibrinogen is the precursor of fibrin, and the primary substrate for stable clot formation. It is depleted after major trauma hemorrhage due to a combination of fibrinogen consumption, breakdown (fibrinolysis) and dilution.[1,2] Observational studies have shown that in severely injured trauma patients, low fibrinogen levels are strongly associated with mortality.[3-6] Many trauma patients have fibrinogen levels on admission below replacement thresholds in major bleeding treatment guidelines.[7] Major hemorrhage protocols (MHPs) guide the delivery of blood components during resuscitation, and aim to deliver a balanced transfusion of red cell, plasma and platelet components in ratios approaching the concentrations found in whole blood.[7-9] However, concentrated fibrinogen products are required to raise levels towards normal and support coagulation in bleeding patients.[6,10-13] Cryoprecipitate is a whole-blood derived, concentrated component that is standard of care for fibrinogen replacement in the US and the UK.

A key question in contemporary trauma resuscitation is whether fibrinogen treatment should be given empirically and in high dose to rapidly correct levels, or later in the course of bleeding as is current practice.[14] The CRYOSTAT-2 trial was designed and powered by the results of the pilot randomized controlled trial (CRYOSTAT),[10] which found early transfusion of cryoprecipitate within a MHP was feasible and restored fibrinogen levels. Our primary hypothesis was that early, empiric and high-dose cryoprecipitate, in addition to standard MHP would improve survival in the first 28 days after hospital admission following injury.

METHODS

Study design and patients

CRYOSTAT-2 was a multicenter, phase 3, interventional, randomized, open-label, parallel-group controlled trial, conducted at 26 Major Trauma Centers in the UK and US. The trial protocol has been previously published in full[15] and available in Supplement 1. Patients were assessed for eligibility by the receiving trauma team leader at each study site. Patients were eligible for the trial if judged to be an adult, aged 16 years or older and have sustained severe injury. Inclusion criteria were the participant to have evidence of active hemorrhage requiring activation of the local MHP and to have started or received at least one unit of any blood component. MHP activation criteria at all centers included systolic blood pressure <90mmHg at any time point. Exclusion criteria were the patient being transferred from another hospital, injuries incompatible with life as assessed by the trauma team leader, or more than three hours had elapsed from the time of injury. A "waiver of consent" was utilized to enroll patients, with written informed consent sought to continue data collection as soon as appropriate after randomization from the participant, or a personal or professional consultee (known as a legally authorized representative in the US). The trial was approved in the UK (17/SC/0164) and granted Section 251 support (19/CAG/0161). In the US the trial was conducted according to the FDA Investigational New Drug regulations (18859) under the Exception from Informed Consent (EFIC) for Emergency Research, 21 CFR 50.24. Community consultation and public disclosure processes were performed before beginning the study. Mortality data at six months and one year was obtained in the UK via a data access request to NHS Digital (US patients at 6 months only via site data collection). This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

Trial Oversight

An independent data monitoring committee reviewed the interim data analysis and monitored patient safety. A Trial Steering Committee provided oversight for the study. The trial is registered at ClinicalTrials.gov (NCT04704869) and ISRCTN14998314. It was performed in accordance with the principles of the Declaration of Helsinki. The authors assume responsibility for the accuracy and completeness of the data and analyses.

Randomization

Randomization and participant allocation was assigned using opaque sealed envelopes in sequence at each site and conducted by study research personnel. The allocation sequence was computer generated, had a varying, undisclosed block size and was stratified by center. Envelopes were securely stored in the Emergency Department (ED) or transfusion laboratory at each site.

Intervention

Patients in both groups received standard treatment (STD) according to the local MHP with a balanced, empiric ratio of red blood cells (RBC), and fresh frozen plasma (FFP). MHPs at participating sites were reviewed by lead trial investigators[14] to ensure consistency.[8,16] Typically, standard MHPs delivered RBC and FFP in 4+4 unit packs, with platelet pools transfused with the second and subsequent packs to achieve a 1:1:1 ratio of RBCs, FFP and platelets. Standard protocols also typically include two pools of cryoprecipitate (4g fibrinogen equivalent), again added to the second and subsequent packs. One prehospital helicopter medical service in the UK utilized a combined "red blood cell and plasma" product and in the US whole blood was available. When transfused both were recorded as one unit of red blood cells and one unit of FFP. In the intervention group (CRYO) patients were to be administered an additional three pools of cryoprecipitate (6g fibrinogen), as early as possible with the aim to start within 90 minutes of admission.

Outcomes

The primary outcome was all-cause mortality at 28 days. Twenty-five pre-specified secondary outcomes included all-cause mortality (including death from bleeding) at 6 hours, 24 hours, 6 months and 12 months from admission; transfusion requirements (RBC, FFP, platelets, cryoprecipitate) at 24 hours; critical care and hospital stay outcomes; destination at discharge; quality of life measures EQ5D-5L[17] and Glasgow Outcome Score (GOS)[18] at discharge or day 28 (whichever came first) and 6 months (eTable 1 in Supplement 3). Safety outcome measures included symptomatic venous or arterial thrombotic events up to day 28 or discharge

(whichever came first). A post-hoc secondary outcome of massive transfusion was added (10+ units of RBC from injury to 24 hours after admission).

Statistical Analysis

The CRYOSTAT-2 trial was designed to detect an absolute mortality difference of seven percent from a baseline mortality of 26%, with 90% power using a five percent level of significance and a two tailed test. The baseline mortality and expected difference between treatment groups chosen for CRYOSTAT-2 was justified by the 28% mortality in the feasibility study CRYOSTAT[10] and was consistent with other recent studies.[19-21] A group sequential design utilizing O'Brien Fleming stopping guidelines [22] required 1530 patients, including interim analyses by the Data Monitoring Committee to assess for evidence of harm or benefit after 500 and 1000 patients had been followed for 28 days. The design meant the significance level to be applied at the final analysis was $p<0.0453$ in order to preserve an overall Type I error throughout the trial of 5%. Allowing for a 2.5% drop-out rate, the initial estimated sample size was 1568 patients. The drop out was higher than anticipated, so this was increased to 4.4%, requiring 1600 patients. The statistical analysis plans (SAPs) are available in Supplement 2. All data were analyzed using SAS version 9.4 (SAS Institute Inc).

Analyses were performed on an intention to treat (ITT) basis and included all randomized patients. Patients discharged prior to 28 days were inferred to be alive at 28 days. Primary outcome analysis was a mixed logistic regression model, adjusted for center. This was supplemented by a risk-adjusted mixed logistic regression analysis to account for other factors associated with outcome. Candidate risk factors were age, sex, type of injury (blunt/penetrating), Injury Severity Score (ISS) admission systolic blood pressure, Glasgow Coma Scale and tranexamic acid administration. An unadjusted analysis (without adjustment for center) was conducted as a sensitivity analysis.

Multiple imputation based on full conditional specification was used to impute values of potential risk adjustment variables. Primary and secondary outcome measures were not imputed, and these were treated as

missing data. A sensitivity analysis assessed the impact of inferring patients discharged alive by randomly changing the outcome to deceased for two percent of inferred cases (a UK national audit of major haemorrhage in trauma showed that 2.2% of patients died between hospital discharge and 1-year[19])

Survival rates after 28-days were estimated using the Kaplan-Meier method and compared using Cox proportional hazards regression. Transfusion requirements were summarized as the median number of units (RBC and FFP), cryoprecipitate pools (5 single donations per pool) or platelet pools (4 whole blood donations per pool/one single donor concentrate) administered from injury to 24-hours post-admission and included the cryoprecipitate transfusion as per the study intervention. Hospital stay, critical care stay and ventilator days were estimated using a competing risks analysis with discharge/extubation as the event and death as the competing risk. A competing risks framework was also used to estimate the cumulative incidence of thrombotic events at day 28. Median EQ-5D-5L values were compared between groups using the Mann-Whitney test. GOS was analyzed using ordinal regression adjusted for center. No corrections for multiple comparisons were made in any of the outcome analyses, and all hypothesis testing was pre-specified in the SAP.

Mortality according to timing of cryoprecipitate administration was analyzed using pre-specified categories of ≤45 minutes from admission, 46 – 60 minutes, 61 – 90 minutes and over 90 minutes. Further pre-defined sub-group analyses of the primary outcome were performed for the variables: head injury (AIS <4 and ≥4); participant sex; age (<70 and ≥70 years); injury type (blunt and penetrating); receipt of cryoprecipitate (or not) in the CRYO group; and location (UK and US patients). A secondary outcome analysis of 6 and 24-hour mortality was also performed for head injury subgroups. A post-hoc subgroup analysis for SBP<90 and ≥90mmHg at hospital arrival was conducted at the request of a reviewer.

A planned per protocol analysis focused on the patients who could have benefitted from the intervention, excluding patients randomized in error, who died within 90 minutes of admission, who did not require blood components after hospital arrival or those with protocol deviations unrelated to cryoprecipitate

administration. This analysis did not exclude those patients only due to non-adherence to the intervention itself, due to potential bias in such exclusions from the lack of a placebo group.

RESULTS

CRYOSTAT-2 enrolled 1604 patients from 25 major trauma centers in the UK (n=1,555) and one in the USA (n=49), between August 2017 and November 2021. On hospital arrival, 805 patients were randomized to receive the standard MHP (STD), and 799 were randomized to receive an additional 3 pools of cryoprecipitate (CRYO). Primary outcome data was missing for 73 patients (most commonly due to withdrawal of consent), leaving 1531 for the ITT analysis (Figure 1). The median time from admission to randomization was 15 minutes. STD and CRYO groups were well matched in baseline clinical characteristics (Table 1) with median age of 39 years and 79% were male. Median ISS was 29, 36% sustained a penetrating injury and 26% had severe head injury (AIS ≥4). On admission, 33% had a systolic blood pressure less than 90 mmHg. Prior to hospital arrival 43% of patients had received a blood component transfusion and 79% had received tranexamic acid (96% of patients received tranexamic acid either prehospital or in hospital).

Cryoprecipitate was administered to 85% (665/785) of patients in the CRYO group and 32% (256/795) in the STD group, within 24 hours of hospital admission. The main reasons for study cryoprecipitate not being transfused were no evidence of active bleeding (n=56), hemostasis had been achieved (n=21) or the patient had died (n=18). Median time from admission to first cryoprecipitate was 68 minutes in the CRYO group vs 120 minutes in the STD group (p<0.0001, eFigure 1 in Supplement 3). In the CRYO group, 68% of patients received their first dose of cryoprecipitate within the study goal of 90 minutes after admission, compared to nine percent in the STD group.

All-cause 28-day mortality for 1531 patients in the ITT analysis was 25.3% in the CRYO group vs 26.1% in the STD group (OR 0.96 (95% CI 0.75-1.23), p=0.74, Figure 2 and Figure 3, RR 0.97 (0.81-1.17)). Mortality was similar between groups at 6 hours (CRYO vs STD: 7.1% vs 8.6%, OR 0.82 (0.58-1.17), p=0.26), 24 hours (CRYO vs STD: 11.2% vs 12.2%, OR 0.91 (0.63-1.31), p=0.61) and at 6 and 12 months (Table 2). The proportion of deaths from bleeding in the first 6 and 24 hours was not different between the two groups. Median time to death from hemorrhage was 191 minutes in the CRYO group vs 86 minutes in the STD group (for those patients who

died from this cause). There was no difference in the causes of death between study groups (eTable 2 in Supplement 3).

There were no observed differences for any secondary outcomes between study groups for 24-hour transfusion requirements (other than cryoprecipitate units), critical care and hospital stays, destination at discharge, EQ5D-5L or GOS (Table 2). There was no difference in any safety outcomes with similar cumulative incidence of thrombotic events (CRYO vs STD: 12.7% vs 12.9%, p=0.89) in both study groups.

The pre-specified analysis of time to cryoprecipitate administration showed that 28-day mortality in the intervention group had an OR 1.45 (95% CI 0.91 – 2.31) for cryoprecipitate transfusion within 45 minutes of arrival; OR 1.16 (0.78 – 1.73) between 46-60 minutes; OR 0.57 (0.38 – 0.87) between 61-90 minutes; and OR 1.00 (0.62-1.60) after 90 minutes (eFigure 2 in Supplement 3). Lack of placebo precluded splitting the STD group by time, and comparison was with whole-cohort mortality. CRYO group patients who received cryoprecipitate very early were more injured and shocked on admission with a trend to higher transfusion requirements (eTables 3 and 4 in Supplement 3). The other pre-specified analysis separated patients in the CRYO group into those who did and did not receive study cryoprecipitate with 28-day mortality of 24.0% vs. 31.7% respectively. There was no statistically significant difference compared to the STD group (received cryoprecipitate, OR 0.90 (0.72-1.13) p=0.36; did not receive cryoprecipitate, OR 1.28 (0.70-2.34) p=0.41).

In the pre-specified subgroup analyses, for 36% of patients with penetrating trauma, 28-day mortality was significantly higher in the CRYO group (CRYO vs STD: 16.2% vs 10.0%, OR 1.74 (1.20-2.51), p=0.006, Figure 2 and Figure 3, RR 1.62 (1.17-2.23)). There were no observed differences in clinical characteristics between the two study groups with this mechanism of injury (eTable 5 in Supplement 3). In contrast, for the 64% of patients with blunt injury, respective mortality rates in the CRYO and STD groups were 30.4% vs 34.8 %, OR 0.82 (0.62-1.09), p=0.16, Figure 2 and Figure 3, RR 0.88 (0.72-1.06)). We did not observe any significant difference in the other subgroup analyses (Figure 3 and eFigure 2 in Supplement 3).

In the per protocol analysis, 170 patients were excluded from the full cohort, principally due to no longer requiring blood transfusion, dying within 90 minutes of arrival, or being randomized in error. Clinical characteristics were similar to the ITT cohort (eTable 6 in Supplement 3) with no difference in all-cause 28-day mortality CRYO: 23.1% vs STD: 22.5% (OR 1.03 (0.77-1.37), p=0.83, RR 1.02 (0.82-1.27)). Secondary outcomes including transfusion requirements, complications and causes of death showed similar patterns to the ITT cohort results with no observed differences other than the expected differences in the amount of cryoprecipitate transfusion. Massive transfusion (RBC 10+ units) was similar between study groups in both ITT, CRYO: 179/785 (23%) vs STD 169/796 (21%) and per protocol analyses, CRYO: 163/727 (22%) vs STD, 146/707 (21%). In ITT sensitivity analyses, the risk adjusted analysis of 28-day mortality included adjustment for GCS, ISS, age and systolic blood pressure and gave a risk-adjusted OR 1.15 (0.93-1.42), p=0.20. Using an unadjusted model and assessing the impact of inferring vital status based on discharge gave consistent results to the primary ITT analysis (Figure 3).

DISCUSSION

In a multicenter, international, parallel group randomized phase 3 trial at 26 major trauma centers, the addition of early and empiric, high dose cryoprecipitate to standard care did not improve clinical outcomes in patients who were severely injured and bleeding. There were no differences in mortality at any time point, nor differences in secondary or safety outcomes.

The CRYOSTAT-2 clinical trial enrolled patients who were severely injured, hypotensive and received substantial blood component transfusions. This was the study population of interest, and the 26% mortality in the STD group was as predicted in the power analysis. CRYOSTAT-2's findings are not consistent with the biological rationale for fibrinogen supplementation in trauma hemorrhage, the results of observational studies[5,6,23,24] and previous pilot or feasibility randomized controlled trials.[10-13,25] It is possible that some patients could have benefited but did not receive cryoprecipitate in time, or at a sufficient dose to restore functional fibrinogen levels. While we aimed for early cryoprecipitate administration, the median time to first transfusion was more than an hour after arrival, reflecting the logistical challenge of preparing and administering a frozen blood component stored in a blood laboratory remote from the patient.

Empiric transfusion of cryoprecipitate could have resulted in a substantial proportion of patients receiving fibrinogen who did not have, and would never develop, hypofibrinogenemia. Fibrinogen has a fundamental importance to clot formation with well described derangements in trauma induced coagulopathy, but empiric treatment has similarly not been found to be of benefit in other bleeding conditions e.g. cardiac surgery[26] and post-partum hemorrhage[27]. High levels of fibrinogen are known to have pro-inflammatory and procoagulant effects[28], which could lead to increased extent and severity of complications such as thrombosis, infarction, and organ dysfunction. We did not observe any overall increase in thrombotic events in this study. However, the observation of increased mortality in the penetrating group and trends in those patients who received cryoprecipitate early, indicate the need for research to fully characterize the safety of fibrinogen in all patients including those who may not have developed low fibrinogen levels. There has not been a specific trauma trial of diagnostic-guided fibrinogen therapy powered for mortality, but clinical trials of precision-guided

coagulation therapy support a targeted approach to administration. Any potential benefit of early supplementation may be only seen in those who are bleeding quickly and most coagulopathic.[11,29,30]

This study has many limitations. First, the multiple challenges in rapidly delivering the intervention leading to variability of timing of cryoprecipitate administration, and an overlap with patients in the STD group receiving it as part of their usual MHP treatment. We were not able to specifically examine the effect of timing of cryoprecipitate administration on outcomes because of the lack of a placebo group. For those patients who died from bleeding, the time to death was prolonged in the CRYO group, by over 100 minutes on average. The clinical effectiveness of cryoprecipitate in these patients may also have been missed due to not all patients being actively bleeding at the time of intervention, and the comparatively long time to administering the intervention. Second, we did not require a research blood sample to be collected for a fibrinogen level immediately prior to cryoprecipitate administration. This was to reduce delays to the intervention and maintain the pragmatic design of the trial to be deliverable at all study sites. From previous studies we would expect approximately a third of all trauma patients to have a fibrinogen concentration below 2g/l on admission, and in up to 75% with trauma-induced coagulopathy.[3,4,10-12,29] However, the prehospital administration of TXA and blood components was high and may reduce the incidence and severity of admission coagulopathy, with relative preservation of fibrinogen levels. In contrast, the longer prehospital times, albeit consistent with other UK trauma hemorrhage trials[20,31], might potentially result in more bleeding, greater consumption of fibrinogen, and therefore a greater expected effect of early replacement therapy. Third, we were unable to open more US sites due to regulatory delays in getting trial approvals, and therefore the study results may not fully reflect US or other international practices and outcomes.

In conclusion, among bleeding trauma patients who required activation of a major hemorrhage protocol, the addition of early and empiric, high-dose cryoprecipitate to standard care did not improve all cause 28-day mortality.

Contributors

RD, NC, HT, AD, LG, JB, SS and KB conceived, designed, and obtained funding for the study. JL, EF, AEv and RS managed and coordinated the project. RD, NC, BC, CW, SS and KB supervised the research. The site Principal Investigators (see Appendix) supervised patient recruitment and with JL, AEv, AEd, LG and EF managed resources at sites including cryoprecipitate and data collation. HT, RS, and SSh were responsible for data curation, formal analysis, and data visualization. RD, KB, SS and HT wrote the original draft, which was then reviewed and approved by all co-authors. All authors had access to the raw data. HT, KB and SS verified the data.

Additional Contributors

We thank the patients and their relatives who supported the trial, principal investigators and other research staff at the study sites, participating blood banks, NHS Blood & Transplant Clinical Trials Unit and Sponsors office, the Trial Steering Committee (Prof Beverley Hunt, Dr Alastair Nimmo, Kathryn Orchard, James Piercy, Dr Jason Sperry), and the Data Monitoring Committee (Prof Timothy Coats, Prof Gavin Murphy, Prof Paul White).

Declaration of Interests

KB and RD receive departmental support from Werfen for coagulation monitoring devices and consumables (not used in this study). RD received departmental support from Hemosonics for coagulation monitoring devices and consumables (not used in this study) and staff costs for a PhD research fellow. RD has received consultancy fees from Werfen and Octapharma for participation in Expert Advisory Board Meetings.

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Role of the Funding Support

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

FIGURE LEGENDS

Figure 1

CRYOSTAT-2 participant flow chart

Abbreviations: STD, Standard group; CRYO, Cryoprecipitate + STD group. Red Blood Cell (RBC). Fresh Frozen Plasma (FFP). Cryoprecipitate (Cryo)

Figure 2

Kaplan-Meier plot of mortality up to 28 days from admission, overall and by injury type, with 95% confidence intervals

Abbreviations: STD, Standard group; CRYO, Cryoprecipitate + STD group.

28-day mortality was analyzed as a binary outcome with odds ratios, 95% CIs and p-values reported in the results and in Figure 3.

Figure 3

Forest plot for main intention to treat and per protocol analyses of the primary outcome, subgroup analyses and sensitivity analyses.

Abbreviations: STD, Standard group; CRYO, Cryoprecipitate + STD group, ITT, Intention to Treat, AIS, Abbreviated Injury Score.

[a] AIS is a severity scoring system that categorizes injury by body region using a 6-point score. A score of 1 describes minor injury and a score of 6 maximal injury. An AIS of 4 or more defines severe injury.

[b] p-value for treatment group term in mixed logistic regression model, adjusted for center

[c] p-value for interaction term between subgroup and treatment group in a mixed logistic regression model, adjusted for center, subgroup and treatment group

[d] p-value for treatment group term in an unadjusted logistic regression model

TABLE 1: DEMOGRAPHICS AND INJURY CHARACTERISTICS

	CRYO group[a] (n=799)	STD group[b] (n=805)
Patients		
Male	618/785 (79)	633/796 (80)
Female	167/785 (21)	163/796 (20)
Age (years)	38 (25-55)	40 (26-55)
Age ≥70 years	71/781 (9)	86/790 (11)
Time from injury to ED arrival (minutes)	75 (55-99)	77 (55-100)
Injuries and physiology on ED arrival		
Blunt injury	495/785 (63)	519/796 (65)
Injury Severity Score[c]	29 (17-43)	29 (18-43)
Head AIS ≥ 4[d]	157/665 (24)	191/664 (29)
Systolic blood pressure (mm Hg)	102 (84-124)	103 (83-126)
Systolic blood pressure <90 (mm Hg)	230/724 (32)	250/738 (34)
Heart rate (per minute)	108 (88-126)	108 (88-127)
In cardiac arrest	12/717 (2)	17/735 (2)
Glasgow Coma Score[e]	14 (3-15)	13 (3-15)
Prehospital interventions		
Red Blood Cell (units)	0 (0-2)	0 (0-2)
Fresh Frozen Plasma (units)	0 (0-1)	0 (0-1)
Crystalloids (ml)	0 (0-250)	0 (0-250)
Colloids (ml)	0 (0-0)	0 (0-0)
TXA administered	615/783 (79)	639/796 (80)

Abbreviations: ED, Emergency Department; MHP, Major Hemorrhage Protocol; AIS, Abbreviated Injury Score; TXA, Tranexamic Acid.

Data are number/total number (%) for categorical variables and median (IQR) for continuous variables.

[a, b] *See figure 1 for descriptions.*

[c] *The score range was 0 to 75. A score greater than 15 indicates major trauma.*

[d] *The score range was 0 – 6. A score of 4 or more indicates severe head injury.*

[e] *The score range was 3 to 15. A score of 8 or less indicates a severe injury that has significantly affected an individual's conscious level.*

TABLE 2: SECONDARY OUTCOMES

	CRYO group[a] (n=799)	STD group[b] (n=805)	p-value
Mortality at 6 hours from admission			
n/N (%)	56/784 (7.1)	68/795 (8.6)	
Absolute difference	-1.5%		
Relative difference	-17.4%		
Odds ratio (95% CI)	0.82 (0.58-1.17)		0.26[i]
Mortality at 24 hours from admission			
n/N (%)	88/783 (11.2)	97/794 (12.2)	
Absolute difference	-1.0%		
Relative difference	-8.2%		
Odds ratio (95% CI)	0.91 (0.63-1.31)		0.61[i]
Estimated mortality rate at 6 months from admission			
Kaplan-Meier estimated % (95% CI)	26.1 (23.2-29.4)	27.3 (24.3-30.7)	
Absolute difference	-1.2%		
Relative difference	-4.4%		
Hazard ratio (95% CI)	0.96 (0.79-1.17)		0.67[j]
Estimated mortality rate at 12 months from admission			
Kaplan-Meier estimated % (95% CI)	26.6 (23.6-30.0)	27.7 (24.6-31.1)	
Absolute difference	-1.1%		
Relative difference	-4.0%		
Hazard ratio (95% CI)	0.96 (0.79-1.17)		0.71[j]

Median (IQR) components transfused over the first 24 hours			
Red Blood Cell (units)	5 (3-9)	5 (3-8)	
Fresh Frozen Plasma (units)	4 (2-8)	4 (2-8)	
Platelets (pools)[c]	0 (0-1)	0 (0-1)	
Cryoprecipitate (pools)[d]	3 (3-3)	0 (0-2)	
Total blood products (units)	12 (7-21)	10 (5-18)	
Massive transfusion n/N (%)	179/785 (23)	169/796 (21)	
Crystalloids (ml)	2000 (700-3500)	1600 (250-3200)	
Colloids (ml)	0 (0-0)	0 (0-0)	
Safety outcomes			
Venous thromboembolism events[e]			
Patients affected – n/N (%)	55/799 (6.9)	**57/805 (7.1)**	
Absolute difference	-0.2%		
Relative difference	-2.8%		
Deep venous thrombosis	20	23	
Pulmonary embolism	38	36	

	CRYO group[a] (n=799)	STD group[b] (n=805)	p-value
Arterial thrombotic events[e]			
Patients affected – n/N (%)	26/799 (3.3)	26/805 (3.2)	
Absolute difference	0.02%		
Relative difference	0.6%		
Stroke	11	11	
Myocardial Infarction	4	4	
Occlusion of other artery	12	11	
All thrombotic events			
Cumulative incidence of thrombotic events at day 28 % (95% CI)	12.7 (10.1-15.6)	12.9 (10.2-15.8)	0.89[k]
Absolute difference	-0.2%		
Relative difference	-1.6%		
Critical care outcomes			
Ventilator days	1 (0-6)	1 (0-7)	0.90[k]
Critical care days (first episode)	4 (1-12)	4 (1-13)	0.85[k]
Hospital outcomes			
Length of stay	11 (3-27)	11 (3-27)	0.88[k]
Destination at discharge			
Home	280/375 (75)	278/374 (74)	
Nursing home/ Rehab facility	9/375 (2)	8/374 (2)	
Other hospital	63/375 (17)	72/374 (19)	
Other[f]	23/375 (6)	16/374 (4)	
Quality of Life			
Median EQ-5D-5L[g] index value at discharge	0.51 (0.26-0.72)	0.50 (0.20-0.73)	0.80[l]
GOS[h] at discharge/day 28			
Low disability	226/705 (32)	221/712 (31)	0.55[m]
Moderate disability	129/705 (18)	129/712 (18)	
Severe disability	155/705 (22)	153/712 (21)	
Persistent vegetative state	21/705 (3)	27/712 (4)	
Death	174/705 (25)	182/712 (26)	

Abbreviations: MHP, major hemorrhage protocol; GOS, Glasgow Outcome Score.

Data are number/total number (%) for categorical variables and median (IQR) for continuous variables.

[a, b] *See figure 1 for descriptions of MHPs.*

[c] *Defined as one pooled platelet concentrate derived from four donations of whole blood, or as one single-donor apheresis platelet concentrate (an equivalent product).*

[d] *Defined as the cryoprecipitate pool derived from five single cryoprecipitate donations.*

[e] *Patients may have experienced more than one event and all events are reported.*

[f] *The most common 'Other' destinations at discharge were mental health facility and police custody.*

[g] *The score range was -0.148 for the worst health state and 0.949 for the best health state.*

[h] *The score used five descriptive terms, defining the global recovery of a participant from injury: low, moderate or severe disability; persistent vegetative state or death. One of these descriptive terms was assigned to each participant at hospital discharge.*

[i] *p-value for treatment group term in mixed logistic regression model, adjusted for center.*

[j] *p-value for treatment group term in Cox regression model with frailty for center*

[k] *p-value for treatment group term in Fine and Gray model*

[l] *p-value from Mann-Whitney test*

[m] *p-value when comparing -2logL of model with and without treatment group term in mixed ordinal regression model, adjusted for center*

REFERENCES

1. Moore EE, Moore HB, Kornblith LZ, et al. Trauma-induced coagulopathy. *Nat Rev Dis Primers.* 2021;7(1):30.
2. Fries D, Martini WZ. Role of fibrinogen in trauma-induced coagulopathy. *Br J Anaesth.* 2010;105(2):116-121.
3. Hagemo JS, Stanworth S, Juffermans NP, et al. Prevalence, predictors and outcome of hypofibrinogenaemia in trauma: a multicentre observational study. *Crit Care.* 2014;18(2):R52.
4. McQuilten ZK, Wood EM, Bailey M, Cameron PA, Cooper DJ. Fibrinogen is an independent predictor of mortality in major trauma patients: A five-year statewide cohort study. *Injury.* 2017;48(5):1074-1081.
5. Inaba K, Karamanos E, Lustenberger T, et al. Impact of fibrinogen levels on outcomes after acute injury in patients requiring a massive transfusion. *J Am Coll Surg.* 2013;216(2):290-297.
6. Rourke C, Curry N, Khan S, et al. Fibrinogen levels during trauma hemorrhage, response to replacement therapy, and association with patient outcomes. *J Thromb Haemost.* 2012;10(7):1342-1351.
7. Rossaint R, Afshari A, Bouillon B, et al. The European guideline on management of major bleeding and coagulopathy following trauma: sixth edition. *Crit Care.* 2023;27(1):80.
8. Cannon JW, Khan MA, Raja AS, et al. Damage control resuscitation in patients with severe traumatic hemorrhage: A practice management guideline from the Eastern Association for the Surgery of Trauma. *The journal of trauma and acute care surgery.* 2017;82(3):605-617.
9. Stanworth SJ, Dowling K, Curry N, et al. Haematological management of major haemorrhage: a British Society for Haematology Guideline. *Br J Haematol.* 2022;198(4):654-667.
10. Curry N, Rourke C, Davenport R, et al. Early cryoprecipitate for major haemorrhage in trauma: a randomised controlled feasibility trial. *Br J Anaesth.* 2015;115(1):76-83.
11. Innerhofer P, Fries D, Mittermayr M, et al. Reversal of trauma-induced coagulopathy using first-line coagulation factor concentrates or fresh frozen plasma (RETIC): a single-centre, parallel-group, open-label, randomised trial. *Lancet Haematol.* 2017;4(6):e258-e271.
12. Curry N, Foley C, Wong H, et al. Early fibrinogen concentrate therapy for major haemorrhage in trauma (E-FIT 1): results from a UK multi-centre, randomised, double blind, placebo-controlled pilot trial. *Crit Care.* 2018;22(1):164.
13. Winearls J, Wullschleger M, Wake E, et al. Fibrinogen Early In Severe Trauma studY (FEISTY): study protocol for a randomised controlled trial. *Trials.* 2017;18(1):241.
14. Marsden M, Benger J, Brohi K, et al. Coagulopathy, cryoprecipitate and CRYOSTAT-2: realising the potential of a nationwide trauma system for a national clinical trial. *Br J Anaesth.* 2019;122(2):164-169.
15. Curry N, Davenport R, Lucas J, et al. The CRYOSTAT2 trial: The rationale and study protocol for a multi-Centre, randomised, controlled trial evaluating the effects of early high-dose cryoprecipitate in adult patients with major trauma haemorrhage requiring major haemorrhage protocol activation. *Transfus Med.* 2022.
16. Glen J, Constanti M, Brohi K, Guideline Development G. Assessment and initial management of major trauma: summary of NICE guidance. *BMJ.* 2016;353:i3051.
17. Herdman M, Gudex C, Lloyd A, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res.* 2011;20(10):1727-1736.
18. Jennett B, Bond M. Assessment of outcome after severe brain damage. *Lancet.* 1975;1(7905):480-484.
19. Stanworth SJ, Davenport R, Curry N, et al. Mortality from trauma haemorrhage and opportunities for improvement in transfusion practice. *Br J Surg.* 2016;103(4):357-365.
20. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. *JAMA.* 2015;313(5):471-482.

21. Cole E, Weaver A, Gall L, et al. A Decade of Damage Control Resuscitation: New Transfusion Practice, New Survivors, New Directions. *Ann Surg.* 2021;273(6):1215-1220.
22. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics.* 1979;35(3):549-556.
23. Stinger HK, Spinella PC, Perkins JG, et al. The ratio of fibrinogen to red cells transfused affects survival in casualties receiving massive transfusions at an army combat support hospital. *J Trauma.* 2008;64(2 Suppl):S79-85; discussion S85.
24. Morrison JJ, Ross JD, Dubose JJ, Jansen JO, Midwinter MJ, Rasmussen TE. Association of cryoprecipitate and tranexamic acid with improved survival following wartime injury: findings from the MATTERs II Study. *JAMA surgery.* 2013;148(3):218-225.
25. Ziegler B, Bachler M, Haberfellner H, et al. Efficacy of prehospital administration of fibrinogen concentrate in trauma patients bleeding or presumed to bleed (FIinTIC): A multicentre, double-blind, placebo-controlled, randomised pilot study. *European journal of anaesthesiology.* 2021;38(4):348-357.
26. Bilecen S, de Groot JA, Kalkman CJ, et al. Effect of Fibrinogen Concentrate on Intraoperative Blood Loss Among Patients With Intraoperative Bleeding During High-Risk Cardiac Surgery: A Randomized Clinical Trial. *JAMA.* 2017;317(7):738-747.
27. Wikkelso AJ, Edwards HM, Afshari A, et al. Pre-emptive treatment with fibrinogen concentrate for postpartum haemorrhage: randomized controlled trial. *Br J Anaesth.* 2015;114(4):623-633.
28. Peng S, Lv K. The role of fibrinogen in traumatic brain injury: from molecular pathological mechanisms to clinical management. *European journal of trauma and emergency surgery : official publication of the European Trauma Society.* 2022.
29. Baksaas-Aasen K, Gall LS, Stensballe J, et al. Viscoelastic haemostatic assay augmented protocols for major trauma haemorrhage (ITACTIC): a randomized, controlled trial. *Intensive Care Med.* 2021;47(1):49-59.
30. Gonzalez E, Moore EE, Moore HB, et al. Goal-directed Hemostatic Resuscitation of Trauma-induced Coagulopathy: A Pragmatic Randomized Clinical Trial Comparing a Viscoelastic Assay to Conventional Coagulation Assays. *Ann Surg.* 2016;263(6):1051-1059.
31. Sperry JL, Guyette FX, Adams PW. Prehospital Plasma during Air Medical Transport in Trauma Patients. *N Engl J Med.* 2018;379(18):1783.