NHS Blood and Transplant

CRYOSTAT-2 EARLY CRYOPRECIPITATE IN TRAUMA

CRYOSTAT-2: longer term follow up

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 (MHP) activation

Early cryoprecipitate in trauma

Queen Mary University of London

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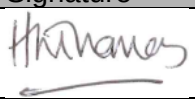
Helen Thomas	Trial Statistician		20/12/2022
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Statistical Analysis Plan

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Statistical Analysis Plan

1. Background and Design

The main characteristics of this trial have been summarised using the CRYOSTAT-2 Protocol v4.0 from 15/02/2022. Please refer to this Protocol for full details. All essential documents for the trial are held in the Trial Master File.

1.1 Trial Summary and Objective

This is 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 (MHP) activation. The study was run and conducted in the UK and USA.

The ultimate goal of this trial is to investigate the effects of transfusing early high dose cryoprecipitate (which is a concentrated source of fibrinogen), to adult trauma patients with severe bleeding within 90 minutes of admission to hospital. This study will evaluate whether early cryoprecipitate (equivalent of 6g fibrinogen replacement) during major traumatic haemorrhage will reduce mortality.

1.2 Patient Eligibility Criteria

Patients are eligible for this trial if:

1. The participant is judged to be an adult (according to local practice, e.g. 16 years or older in UK) and has sustained severe traumatic injury
2. The participant is deemed by the attending clinician to have on-going active haemorrhage
   AND REQUIRES:
3. Activation of the local major haemorrhage protocol for management of severe blood loss
   AND HAS STARTED or HAS RECEIVED:
4. At least one unit of any blood component

A patient will not be eligible for this trial if they fulfil one or more of the following criteria:

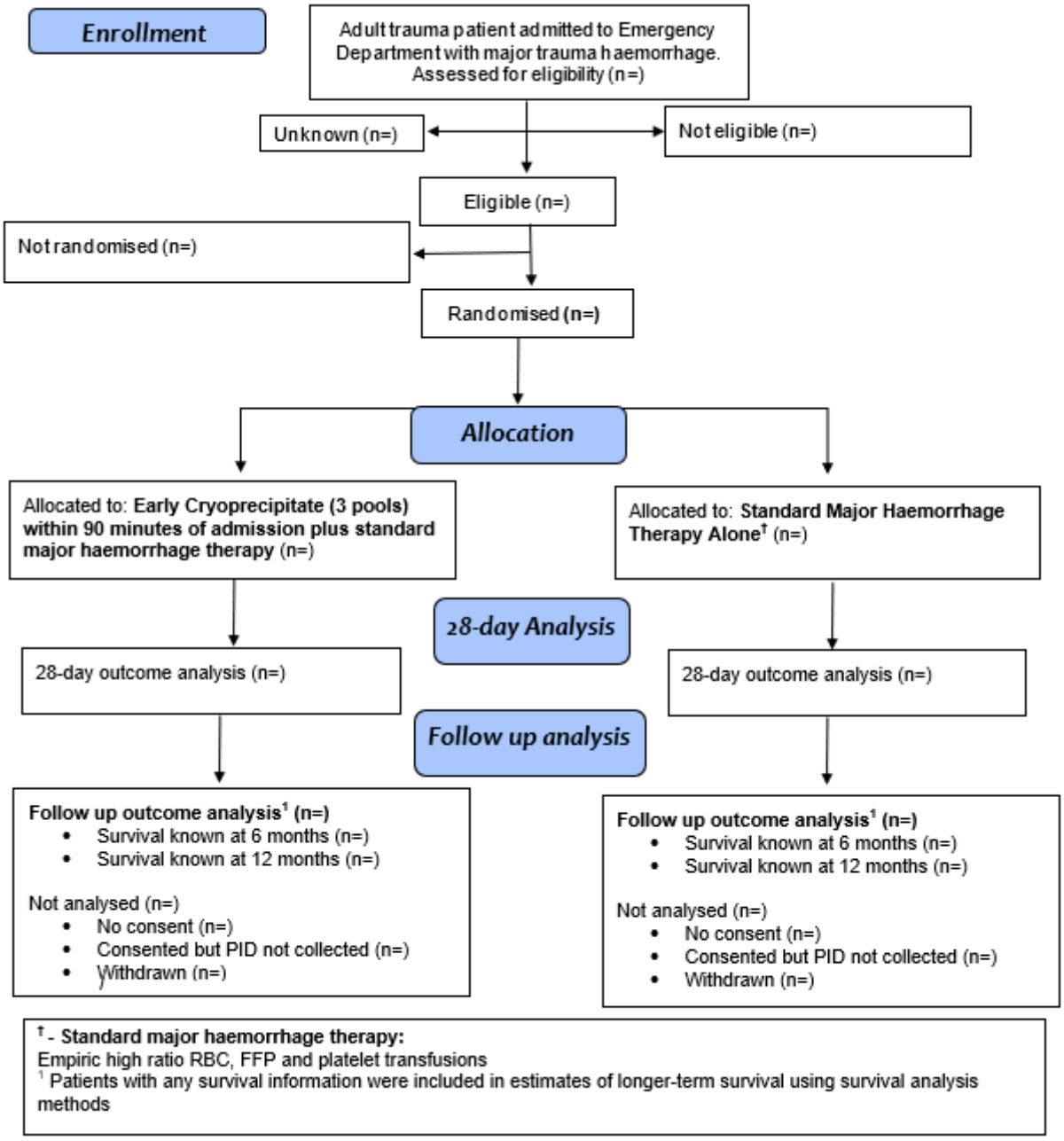
1. The participant has been transferred from another hospital
2. The trauma team leader deems the injuries incompatible with life
3. More than 3 hours have elapsed from the time of injury (taken as time of the 999 call if unknown by medical team).

1.3 Trial Intervention

Intervention arm:
Early intravenous infusion of cryoprecipitate (3 pools of cryoprecipitate – the equivalent to 15 single units of cryoprecipitate or 6g fibrinogen), within 90 minutes of admission to hospital in addition to standard major haemorrhage therapy.

Comparator arm:
Major haemorrhage therapy alone.

Figure A Summary of trial entry, randomisation and treatment



1.4 Randomisation and Blinding Procedures

Participants were allocated in a 1:1 ratio to either intervention (early cryoprecipitate) or comparator (standard MHP) arm. The allocation sequence was produced by the trial statistician using SAS statistical software. It had a varying block size that was not disclosed, and was stratified by centre.

Allocation cards were prepared independently and in advance by the CTU and provided to participating sites. Each envelope contained a randomisation number and the allocated treatment. Envelopes were opened in sequential order.

Members of the trauma team and study research personnel enrolled participants and assign them to the randomised treatment.

1.5 Sample size calculation

See 28-day statistical analysis plan (CRYOSTAT2_SAP_v2.0_22_05_20) for details.

2. Data Handling

2.1 CRF descriptions and data collection schedule

For the longer term follow up data, patients' health records enrolled at Major Trauma Centres (MTCs) in England were flagged for death up to 1 year from date of admission via a data access agreement with NHS Digital (who hold the mortality data on behalf of the Office for National Statistics (ONS)). Flagging data (including patient name, NHS, date of birth, sex and postcode) were collected for those with consent or Section 251 exemption, and submitted to NHS Digital on a periodic basis for the receipt of mortality data including date and cause of death (if applicable). A small number of participants in Wales and Northern Ireland could not be submitted due to NHS Digital or Section 251 restrictions. There were no participants in Scotland.

Similarly, where the same flagging data were collected, this was shared with the Trauma Audit and Research Network (TARN) for the provision of 6-month quality of life data.

In the US, 6-month survival and quality of life data were collected on a study CRF by the trial co-ordinators, and entered onto the MACRO study database. 12-month data is not available for US participants.

Timepoint*	***On arrival at hospital***	***Enrolment***	***Post randomisation***					
		T_0	T_1	T_2	T_3	T_4	T_5	T_6
ENROLMENT								
Eligibility Screen		*X*						
Emergency waiver		*X*						
Randomisation/allocation		*X*						
INTERVENTION			◄►					
Cryoprecipitate (3 pools, 15 units) in addition to MHP			*X*					
Major haemorrhage protocol (MHP) alone			*X*					
ASSESSMENTS								
Participant characteristics		*X*						
Clinical assessment		*X*						
TXA administration			*X*					
RBC, FFP, cryo, platelets (including given pre-hospital)	*X*				*X*			
Mortality			*X*	*X*	*X*	*X*	*X*	*X*
Serious Adverse Events					*X*	*X*		
Participant destination at discharge						*X*		
Glasgow Outcome Score						*X*	*X*	
EQ5D-5L						*X*	*X*	
**Timepoints are:*	*T_0 – Enrolment & allocation of early cryoprecipitate, must be within 90 minutes of arrival at hospital* *T_1 – cryoprecipitate administered, must be started within 90 minutes of arrival at hospital* *T_2 – 6 h (± 1 h) from arrival at hospital*							

	T_3 - 24h (± 4 h) from arrival at hospital *T_4 – date of discharge or day 28 (± 4 days) from arrival at hospital whichever is the sooner* *T_5 - 6 months (± 14 days)* *T_6- ≥12 months (mortality data post discharge to be captured by flagging with the ONS, for English sites only)*

2.2 Procedures for recording and reporting outcomes

For the US site, the Principal Investigator has overall responsibility for data collection on a designated paper Case Report Form. Participant data will be sent to NHSBT CTU for data entry onto the trial database, MACRO™, a commercially available FDA 21 CRF Part 11 compliant clinical trial database system produced by Elsevier. Data will be recorded on a paper CRF which was compiled by the CTU. Three participant identifiers (Participant ID allocated at randomisation, initials and site number) will be captured on the CRF. Prime responsibility for the complete collection of data for the centre will reside with the local Principal Investigator but may be delegated (for example to a research nurse). Overall responsibility for collating data will reside with the Trial Manager.

For English sites, centralised mortality data will be captured via the ONS, specifically at six- and twelve-months. Participants' health records enrolled at MTCs in England will be flagged for death up to 1 year from date of admission. In the unlikely event that mortality data are not available, or a participant is considered lost to follow up, the research team at the study centre will be asked to contact the participant's GP to confirm their survival status, and if appropriate, contact the participant. Participants were fully informed of this mechanism and asked to consent to being contacted in such circumstances via written informed consent.

Quality of life data at hospital discharge and 6 months from the time of injury (± 14 days) will be collected via TARN.

2.3 End-point Review Panel assessments/SAE Review

For this follow up analysis, causes of death provided by NHS Digital will be categorised into one of the groups used on the CRFs by at least one clinical member of the central trial team in a blinded manner. See table 6 for the groupings.

2.4 Other assessments

N/A

2.5 Trial Data Management and Verification

The trial database was locked for the 28-day analysis and will be unlocked according to the processes described in (MPD997) to allow entry of the 6-month CRF data from the US. The database will then be re-locked. Quality control of this additional data entered and data cleaning will be performed by the trial data manager and is detailed in the Data Management Plan v1.2 dated 3 May 2022. This will include performing range, data completeness and consistency checks. Once this stage is finished, the trial dataset will be declared frozen and exported from the MACRO database for final data review and validation checks by a statistician, who will raise data queries with the trial manager or data manager. Once the trial statistician, data manager, and trial manager are satisfied that all

queries have been resolved, the database will be re-locked. The locked database will be extracted into a statistical software package and used for final analysis.

3. Detailed Analysis Plan

3.1 Interim analysis and sample size re-estimation (if applicable)

See 28-day statistical analysis plan (CRYOSTAT2_SAP_v2.0_22_05_20).

3.2 Analysis principles

This analysis plan only includes analyses of outcomes at 6 and 12 months (secondary outcomes (1) and (5) in protocol v4.0). Refer to the 28-day statistical analysis plan for the analysis of outcomes up to 28 days from admission to hospital (CRYOSTAT2_SAP_v2.0_22_05_20).

The only analysis population will be intention to treat. This will include all randomised participants on whom values of a response variable has been obtained. The data will be analysed according to the treatment arm to which the participant was randomised.

Participants who did not meet all the inclusion criteria and/or met at least one of the exclusion criteria, or those who were co-enrolled in other trials without approval, will be considered as randomised in error. Participants who were randomised to the early cryoprecipitate arm, and did not receive at least 3 pools of cryoprecipitate, or did not start their first infusion of cryoprecipitate within 90 minutes of admission or more than 3 hours from injury will be considered as protocol deviations, together with any other significant deviations from the protocol. Participants randomised in error, participants with protocol deviations and those withdrawn will be included in the intention to treat analysis where possible. Participants lost to follow up will also be included in the analysis where possible. Those who consented to and were randomised in the trial will be analysed, even if they did not receive the assigned intervention, to understand reasons for failure to complete the assigned strategy.

6-month data are only available in the US where the participant could be contacted, or vital status otherwise ascertained. 6 and 12-month data are only available in England for those where the participant gave informed consent to linkage, or where the participant was covered by the Section 251 exemption obtained during the study. For those with consent, patient identifiable data to undertake the linkage also needed to be provided by the site. Some linked participants had not reached the 12-month timepoint at the time of final linkage, but latest available survival information will be used.

Survival analysis methods will be used for all analyses of 6- and 12-month survival. This means all participants with survival data at any time can be included, and censored at last known survival where relevant. This approach maximises use of available data, rather than restricting the analysis to only those where 6- or 12-month survival was definitively known.

A CONSORT diagram will be presented to show how participants progressed through the trial, including the numbers of participants included in follow-up survival analysis (see Figure 1). The number of participants with survival data definitively known at 6 months, and at 12 months will also be provided. The number of participants who didn't provide consent for longer term follow up, provided consent but patient identifiable data (PID) was not collected, or withdrew from the longer term follow up will be presented.

Outcomes will be presented by treatment arm as numbers, percentages, means and medians as appropriate. Standard deviations and interquartile ranges will also be presented to describe the uncertainty in the estimates and the spread of the data. Where there is interest in differences between treatment arms, statistical tests will be performed, and these will be indicated in the tables. This study has been powered to detect a difference between treatment arms in the primary outcome of 28-day all-cause mortality only. There may not be sufficient power to detect differences between arms for the longer term outcomes.

Follow up survival data up to 12 months will be taken from NHS Digital data (assuming the absence of a death record means the patient was alive 2 weeks before the trace was conducted), or from the study CRF data where no linkage was possible. Only participants who are missing survival data at all time points will be excluded from the analysis. The survival analyses will censor participants at their last known survival time, if no death recorded.

Unadjusted survival rates will be estimated using the Kaplan-Meier method and presented with confidence intervals. The primary analysis will be a Cox regression model with adjustment for centre through the inclusion of a frailty term, as centre is used in the randomisation process. The hazard ratio for treatment will also be presented after adjustment for other patient specific factors. For any Cox regression models, p-values will be obtained by comparing -2 x log-likelihood value for models with and without the treatment term using a chi-squared distribution. The proportional hazards assumption between treatment arms will be checked using a log-cumulative hazard plot and Schoenfeld residuals.

All descriptive statistics will be unadjusted. All tests will be two-sided and p-values of less than 0.05 will be considered as statistically significant. All hazard ratios will be presented for the early cryoprecipitate arm relative to the standard MHP arm. A ratio which is greater than 1 indicates that the hazard of the event is greater in the early cryoprecipitate arm. Two-sided 95% confidence intervals will be presented with all ratios. P-values will be reported to four decimal places with p-values less than 0.0001 as <0.0001. The statistical package SAS will be used to conduct analyses.

Multiple comparisons will be performed, and this may increase the probability of observing a statistically significant result by chance. No adjustments will be made to account for multiple testing as all analyses have been pre-specified.

3.3 Analysis of secondary outcome measures

For each of the secondary outcome measures, the data presented and any statistical tests performed are described below. In all cases data will be presented for each arm of the trial separately, and overall.

All-cause mortality (including death from bleeding) at 6 months and 12 months from admission

All mortality related analyses will be produced for both 6-month and 12-month endpoints.

The proportion of participants who died from any cause at 6 months and 12 months from admission will be estimated by the Kaplan-Meier (KM) method and compared by treatment arm using Cox regression analysis with a frailty term for centre. Participants withdrawn or lost to follow up before the endpoints will be censored at the time they withdrew/were lost to

follow up. Participants with no survival data from any source will be summarised, but not included in the survival analyses. Hazard ratios and p-values from the Cox model only adjusted for centre will be presented and this will be the primary analysis comparing 6 and 12-month mortality between arms. An unadjusted KM plot up to 12 months from admission by treatment arm will be presented. Numbers at risk will be included below the plot.

A further Cox regression analysis for death within 6 months and 12 months will separately be performed, to assess the effect of treatment arm after adjusting for other relevant factors. The factors to be considered for inclusion in the models are: age, type of injury (blunt/penetrating), systolic blood pressure (SBP), Glasgow Coma Scale (GCS), early bolus of tranexamic acid (TXA) (pre-hospital or in the Emergency Department), Injury Severity Score (ISS) and sex. Factors will only be added to the Cox regression model if they are statistically significant in improving the model fit at the 10% level. Frailty for centre will be included in the model. Multiple imputation based on full conditional specification will be used to impute any missing values for these potential risk adjustment factors. The set of variables used in the multiple imputation models will be: last known vital status, age, type of injury (blunt/penetrating), SBP, GCS, early bolus of TXA, ISS, sex, heart rate and treatment arm.

The adjusted hazard ratio for death at each endpoint for the early cryoprecipitate arm, relative to the standard arm, with 95% confidence interval, following adjustment for any significant participant factors and centre will be presented (see tables 2 and 3). For the analysis including adjustment for participant factors, the adjusted hazard ratio for death at each endpoint, 95% confidence interval and p-value will be presented for each factor in the Cox regression model (see tables 4 and 5).

Cause of death will be summarised for all patients that died, by treatment arm, with counts and percentages (for each endpoint).

Quality of life measures: EQ5D-5L at discharge and 6 months

This section will replicate the initial quality of life section of the 28-day analysis plan but will now also include the measures at the 6 month follow up. The below description therefore refers to both the analysis looking at discharge or day 28 where alive, and at the 6 month follow up.

For the EQ-5D-5L descriptive systems questionnaire, the number and percentage of participants who have completed the index value questions and health today question will be presented by arm. In addition, median (interquartile range (IQR)) and mean (standard deviation) index value will be calculated, along with Cohen's d and a 95% confidence interval for the standardised difference in mean index values between arms. The index value allows the five health dimensions to be converted into a single numeric measure, with higher values reflecting better health. 1 relates to 'perfect health' and 0 to 'dead', but some index values can be negative. The Mann-Whitney test will be used to examine whether the difference in median index values is significant between the two treatment arms. For EQ Visual Analogue Scale (VAS), median (IQR) and mean (standard deviation) self-evaluated health score will be calculated, along with Cohen's d and a 95% confidence interval for the standardised difference in mean self-evaluated health score between arms. This score has a range of 0-100, with higher values reflecting better health. The Mann-Whitney test will be used to examine whether the difference in median self-evaluated health score is significant between the two treatment arms.

3.4 Procedures for handling Missing Data

Any missing secondary outcome data will be summarised. Secondary outcome measures will not be imputed for these analyses and these will be treated as missing data and excluded from the relevant analyses. If outcome data is missing for more than 25% of participants, outcomes will not be reported.

Section 3.3 describes the multiple imputation that will be conducted for the secondary risk-adjusted analysis of survival.

4. Data Analysis Tables

Data analysis will be based on the following tables. Further tables/rows/columns may be added in the Data Analysis Report if deemed necessary.

4.1 Missing Data tables

Table 1 Missing follow up outcomes data table – n/N (%)

Outcome	Std MHP arm (n=)	Early cryo arm (n=)	Overall (n=)
Missing data for vital status			
Missing cause of death (for those who died within 12 months)			
Missing data for EQ-5D-5L questionnaire needed for index value, completion at discharge or day 28 (where alive)			
Missing data for self-evaluated health score on EQ-5D-5L at discharge or day 28 (where alive)			
Missing data for EQ-5D-5L questionnaire needed for index value, completion at 6-month follow up (where alive at last follow-up)			
Missing data for self-evaluated health score on EQ-5D-5L at 6-month follow up (where alive at last follow-up)			

4.2 Secondary Outcome table(s)

Table 2 All-cause mortality at 6 months by arm

Outcome	Std MHP arm (n=)	Early cryo arm (n=)	Overall (n=)	P-value
Estimated mortality rate at 6 months from admission[1] - % (95% CI)				
Hazard ratio[2] (95% CI)				
Hazard ratio also adjusted for participant factors[3] (95% CI)				
Participants with missing survival data at all time points – n/N (%)				

[1] Unadjusted Kaplan Meier estimate

[2] Early Cryo arm relative to Standard arm, adjusted for centre, p-value for treatment term in Cox regression model.

[3] Early Cryo arm relative to Standard arm adjusted for centre and significant participant factors.

Note: Participants for whom vital status was not available were not included in this analysis in addition to x participants excluded due to x, y and z.

Table 3 All-cause mortality at 12 months by arm

Outcome	Std MHP arm (n=)	Early cryo arm (n=)	Overall (n=)	P-value
Estimated mortality rate at 12 months from admission[1] - % (95% CI)				
Hazard ratio[2] (95% CI)				
Hazard ratio also adjusted for participant factors[3] (95% CI)				
Participants with missing survival data at all time points – n/N (%)				

[1] Unadjusted Kaplan Meier estimate

[2] Early Cryo arm relative to Standard arm, adjusted for centre, p-value for treatment term in Cox regression model.

[3] Early Cryo arm relative to Standard arm adjusted for centre and significant participant factors.

Note: Participants for whom vital status was not available were not included in this analysis in addition to x participants excluded due to x, y and z.

Table 4 Risk-adjusted model for all-cause mortality at 6 months

Risk Factor	Hazard ratio[1] (95% CI)	P-value
Risk factor A		
Risk factor B		
.		
.		
.		
Early cryoprecipitate arm		

[1]Adjusted hazard ratio from Cox regression model, also adjusted for centre

Table 5 Risk-adjusted model for all-cause mortality at 12 months

Risk Factor	Hazard ratio[1] (95% CI)	P-value
Risk factor A		
Risk factor B		
.		
.		
.		
Early cryoprecipitate arm		

[1]Adjusted hazard ratio from Cox regression model, also adjusted for centre

Table 6 Causes of death for all-cause mortality at 6 months - n/N (% of those who died within 6 months)

Cause of death	Std MHP arm (n=)	Early cryo arm (n=)	Overall (n=)
Total reported deaths within 6 months – n/N (% of those randomised)			
Multi-organ failure			
Multiple injury			
Myocardial infarction			
Pulmonary embolism			
Sepsis			
Stroke			
Traumatic brain injury			
Uncontrolled bleeding			
Other			
All			
Note: Due to incomplete linkage, the numbers in this table are likely to be underestimates			

Table 7 Causes of death for all-cause mortality at 12 months - n/N (% of those who died within 12 months)

Cause of death	Std MHP arm (n=)	Early cryo arm (n=)	Overall (n=)
Total deaths within 12 months– n/N (% of those randomised)			
Multi-organ failure			
Multiple injury			
Myocardial infarction			
Pulmonary embolism			
Sepsis			
Stroke			
Traumatic brain injury			
Uncontrolled bleeding			
Other			

All			
Note: Due to incomplete linkage, the numbers in this table are likely to be underestimates			

Table 8 Quality of life at discharge and 6 months after admission

	At discharge or day 28 where alive				At 6-month follow up			
Outcome	**Std MHP arm (n=)**	**Early cryo arm (n=)**	**Overall (n=)**	**P-value**	**Std MHP arm (n=)**	**Early cryo arm (n=)**	**Overall (n=)**	**P-value**
Participants who completed EQ-5D-5L index value questions - n/N (%)								
Participants who completed EQ-5D-5L health today question - n/N (%)								
Median (IQR) index value[1]								
Mean (SD) index value								
Cohen's d for index value (95% CI)								
Median (IQR) self-evaluated health score[1]								
Mean (SD) self-evaluated health score								
Cohen's d for self-evaluated health score (95% CI)								

[1] P-value for Mann-Whitney test

4.3 Figures

Figure	Description
Figure 1	Participant flow chart (CONSORT diagram)
Figure 2	Kaplan-Meier survival plot up to 12 months from admission by treatment arm, intention to treat

5. Statistical Analysis Plan Amendments

Revision History:

Version	Author	Date	Reason for revision

6. References

NHSBT CTU MPD998 v4.1 Statistical Analysis and Reporting