COPTIC: Coagulation and platelet laboratory testing in cardiac surgery
A prognostic study of associations between post-operative test results and subsequent bleeding

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List of abbreviations

Acronym	Details
AKI	Acute kidney injury
AUC	Area under the curve
CCB	Clinical concern about bleeding
CI	Confidence interval
CICU	Cardiac intensive care unit
CNS	Central nervous system
CPAP	Continuous positive airway pressure
CRF	Case report form
CTEU	Clinical Trials and Evaluation Unit
eGFR	estimated Glomerular Filtration Rate
EPT	Early pro-haemostatic treatment
ETP	Endogenous thrombin potential
FBC	Full blood count
GI	Gastrointestinal
HArVeST	A Randomised Controlled Trial to Assess the Extent of Intimal Hyperplasia and Atherogenesis in Bypass Vein Grafts Following Different Surgical Preparation Techniques
HB	Haemoglobin
HCT	Haematocrit
IPF	Immature platelet fraction
IQR	Inter quartile range
MI	Myocardial infarction
MPV	Mean platelet volume
NSTS	NHS Strategic Tracing Service
OR	Odds ratio
PASPORT	Patient-specific cerebral oxygenation monitoring as part of an algorithm to reduce transfusion during heart valve surgery: a randomised controlled trial
PATS	Patient Analysis and Tracking Systems
ProMPT	Propofol cardioplegia for Myocardial Protection Trial
PROTECTION1	Pulmonary protection with low frequency ventilation during cardiopulmonary bypass: a prospective randomised study
RBC	Red blood cells
rFVII	Recombinant human coagulation factors VII
ROTEM	Point-of-care whole blood haemostasis analyser
SAP	Statistical analysis plan
SD	Standard deviation
STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
TEG	Thromboelastogram
TITRe2	A multi-centre randomised controlled trial of Transfusion Indication Threshold Reduction on transfusion rates, morbidity and healthcare resource use following cardiac surgery
UHBristol	University Hospitals Bristol NHS Foundation Trust

Acronym	Details
VeRDiCT	Preoperative Volume Replacement vs. usual care in Diabetic patients having CABG surgery: a randomised controlled Trial
WBC	White blood cells

Definitions

Coagulopathy	Any abnormality of blood clotting (coagulation factors or platelets)
Clinical concern about bleeding	Excessive actual blood loss **or** non-routine pro-haemostatic intervention before excessive blood loss has occurred **or** coagulopathic bleeding identified at re-operation.
$P2Y_{12}$ blocker	Clopidogrel or Prasugrel
Post-operative test results	Laboratory test results from Multiplate, ROTEM or TEG

1. INTRODUCTION

1.1 Scope

This statistical analysis plan (SAP) covers the analyses of the Multiplate, ROTEM and TEG results for the post-operative blood samples collected for the COPTIC study (part of Study B). This SAP does not cover the Health Economics analyses which will be covered in separate documentation.

Any changes made to this SAP after approval must be clearly justified and documented as an amendment at the end of this document. The SAP should then be re-approved.

1.2 SAP document approval

The study statistician should authorise the SAP.

1.3 Skeleton tables and figures

Throughout this document references are made to any skeleton tables and figures to be used in the reporting of the study (e.g. **Figure F1** or **Table T1**). Such tables and figures can be found in **Appendix A** of this document, and are intended as a guide for study reporting. Final versions of the tables/figures may differ: tables may be combined, and/or their layout or numbering may differ. However the content should be consistent with **Appendix A.**

2. STUDY BACKGROUND AND OBJECTIVES

2.1 COPTIC study background

Cardiac surgery is associated with complex abnormalities in the blood coagulation (clotting factors and platelets) that are caused by patients' characteristics, drug treatments and the mechanical interventions that are necessary for surgery. These include pre-operative abnormalities such as low platelet function caused by exposure to anti-platelet drugs, and intra-operative abnormalities such as dilution or inactivation of blood clotting factors and platelets, reduced clotting factor function because of heparin and increased clot breakdown. The combination of pre-operative and intra-operative abnormalities in blood coagulation may lead to increased bleeding after cardiac surgery that requires treatment with blood components (e.g. fresh frozen plasma, platelet transfusion) and/or red cell transfusion. Bleeding, blood component and red cell transfusion are independently associated with adverse clinical outcomes after cardiac surgery.

The COPTIC study will investigate how the results of improved testing for abnormalities in blood coagulation abnormalities both before surgery (COPTIC Study A) and after surgery (COPTIC Study B) can be used to benefit patients by improving diagnosis and appropriate treatment with blood components.

2.2 Overview of COPTIC study design

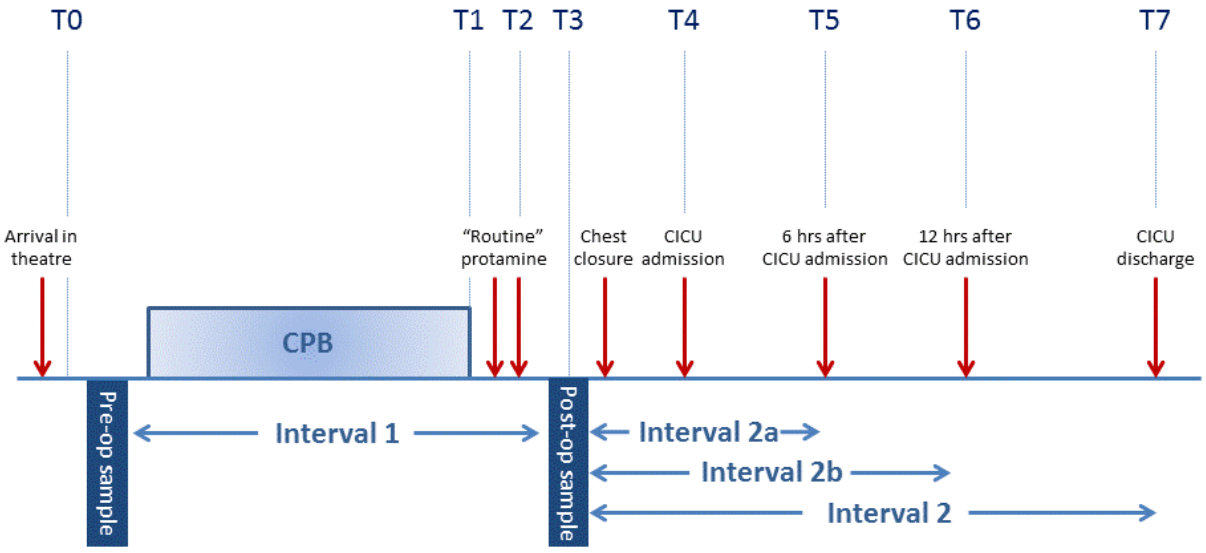
COPTIC is a prospective observational study of patients undergoing cardiac surgery at the Bristol Heart Institute.

i. All participants undergo standard pre-operative, anaesthetic, surgical and post-operative care according to existing protocols.
ii. Blood samples are obtained in the operating theatre at the following time-points:

a. Immediately before induction of anaesthesia (*pre-operative sample; 9.5mls*)
b. Immediately after reversal of heparin anticoagulation (*post-operative sample; 18.5mls*)

Data are collected until 12 hours after admission to Cardiac intensive care unit (CICU). The study timings are shown in Figure 1.

Figure 1: Timeline for COPTIC study



T0: time of pre-op sample
T1: cessation of CPB
T2: administration of final routine dose of protamine
T3: time of post-op sample
T4: time of arrival in CICU
T5: 6 hours after arrival in CICU
T6: 12 hours after arrival in CICU
T7: CICU discharge

Interval 1 time interval between pre-op and post-op samples[T3-T0]
Interval 2a post-op sample to 6 hours after admission to CICU [T5-T3]
Interval 2b post-op sample to 12 hours after admission to CICU [T6-T3]
Interval 2 time interval between post-op sample and CICU discharge[T7-T3]

This SAP refers to the analysis of post-operative Multiplate test results, ROTEM test results and TEG test results (part of Study B). A separate analysis plan will be written for the analyses of the other laboratory results from post-operative samples (also Study B).

2.3 Study objectives

COPTIC Study B examines the relationship between post-operative abnormalities in blood coagulation and 'clinical concern about bleeding' (CCB) after cardiac surgery (see section 5.1 for the definition of the primary outcome). Although numerous abnormalities in both clotting factors and platelets have been reported in patients before cardiac surgery, the most prevalent and clinically important abnormalities are likely to be in platelets. This is because anti-platelet drugs such as aspirin and the $P2Y_{12}$ blockers, clopidogrel or

prasugrel, are prescribed widely to patients before cardiac surgery and markedly reduce platelet function.

Approximately 50% of cardiac surgery patients receive aspirin alone up to the day of surgery and approximately 25% receive dual therapy with aspirin plus a $P2Y_{12}$ blocker. For the dual-therapy sub-group, current practice guidelines suggest withdrawal of the $P2Y_{12}$ blocker approximately 5 days before cardiac surgery but continuation of aspirin up to the day of surgery. In practice, the duration of withdrawal varies between 0 and 7 days because of clinical operational constraints. We hypothesise that exposure to aspirin alone or exposure to aspirin + $P2Y_{12}$ blocker is associated with more CCB than no exposure to anti-platelet drugs. We also hypothesise that in the dual-therapy sub-group, a short time of withdrawal of $P2Y_{12}$ blocker is associated with more CCB than a long time of withdrawal.

It is already known that anti-platelet drugs cause abnormal results in some laboratory tests of platelet function. Some other factors may also influence platelet function tests. It is unknown whether any laboratory test or combination of tests of platelet function can be used to predict CCB. The overall aim of the COPTIC Study B is to estimate the association between the results from a panel of laboratory tests of platelet function and count performed after cardiac surgery and CCB in all patients. Since anti-platelet drugs have marked effect of some platelet laboratory tests, we will also estimate the association between test results and CCB in patient subgroups (no anti-platelet drugs vs. aspirin alone vs. aspirin plus $P2Y_{12}$ blockers). We will also estimate the association between test results and duration of withdrawal of $P2Y_{12}$ blockers and the relationship between duration of withdrawal of $P2Y_{12}$ blockers and CCB. Other pre-operative, intra-operative and post-operative factors (up to T6) will be considered as potential confounders of the association between platelet test results and CCB. Estimates of association will be used to generate a parsimonious predictive model of the probability of CCB based on post-operative platelet test results and other factors.

The specific study objectives are as follows:

1. In all patients:
   (a) to describe the distributions of post-operative test results ('primary predictors' and 'secondary predictors', see section 2.6).
   (b) to examine the relationship between post-operative test results and the primary clinical outcome of CCB (see section 5.1).

2. In the patient sub-groups *no anti-platelet drugs*, *aspirin alone* and *aspirin+ $P2Y_{12}$ blockers*:
   (a) to describe the distributions of post-operative test results ('primary predictors' and 'secondary predictors')
   (b) to examine the relationship between i) post-operative test results and ii) the primary clinical outcome of CCB (see section 5.1).

3. In the patient sub-group *aspirin+ $P2Y_{12}$ blockers:*
   (a) to describe the distributions of duration of withdrawal of $P2Y_{12}$ blocker

(b) to examine the relationship between the duration of withdrawal of $P2Y_{12}$ blocker and post-operative test results.

(c) to examine the relationship between the duration of withdrawal of P2Y12 blocker and the primary clinical outcome of CCB.

2.4 Primary outcome

The primary outcome for COPTIC Study B is clinical concern about bleeding (CCB; see section 5.1 for the definition).

2.5 Secondary outcomes

Secondary outcomes for COPTIC Study B are:

(a) Whether red cell transfusion was given and the quantity of red cells transfused.

(b) The quantities of non-routine pro-haemostatic treatments (see section 5.2 for the definition).

(c) Serious post-operative complications (see section 5.2 for definition).

2.6 Laboratory predictors

Although approximately 30 post-operative test results have been recorded in the COPTIC Study B, these may be sub-classified according to likelihood of an association with the primary outcome of CCB (objectives 1 and 2):

2.6.1 Primary laboratory predictors (test results considered likely to predict CCB)

Test result	Laboratory test and platform	Likely predictor of CCB
Platelet count	FBC (XE-2100 cell counter)	LOW test result
Multiplate ADP test AUC	Multiplate	LOW test result
Multiplate AA test AUC	Multiplate	LOW test result
Multiplate TRAP test AUC	Multiplate	LOW test result
Multiplate ADR test AUC	Multiplate	LOW test result
ROTEM intem CT	ROTEM	HIGH test result
ROTEM intem alpha	ROTEM	LOW test result
ROTEM intem mcf	ROTEM	LOW test result
ROTEM intem ml	ROTEM	HIGH test result
ROTEM intem maxV	ROTEM	LOW test result
ROTEM intem maxV-t	ROTEM	HIGH test result
ROTEM extem CT	ROTEM	HIGH test result
ROTEM extem alpha	ROTEM	LOW test result
ROTEM extem mcf	ROTEM	LOW test result

ROTEM extem ml	ROTEM	HIGH test result
ROTEM extem a10	ROTEM	LOW test result
ROTEM extem maxV	ROTEM	LOW test result
ROTEM extem maxV-t	ROTEM	HIGH test result
ROTEM fibtem mcf	ROTEM	LOW test result
ROTEM fibtem a10	ROTEM	LOW test result
ROTEM extem mcf - fibtem mcf	ROTEM	LOW test result
ROTEM intem CT - heptem CT	ROTEM	HIGH test result
TEG CK rmin	TEG	HIGH test result
TEG CK angledeg	TEG	LOW test result
TEG CK ma	TEG	LOW test result
TEG CK ly60	TEG	HIGH test result
TEG CK rmin – CKH rmin	TEG	HIGH test result

2.6.2 Secondary laboratory predictors (test results considered less likely to predict CCB)

Test result	**Laboratory test and platform**	**Likely predictor of CCB**
Haemoglobin concentration (HB)	FBC (XE-2100 cell counter)	LOW test result
Haematocrit (HCT)	FBC (XE-2100 cell counter)	LOW test result
White blood cells (WBC)	FBC (XE-2100 cell counter)	LOW test result
Absolute neutrophils	FBC (XE-2100 cell counter)	LOW test result
Mean platelet volume (MPV)	FBC (XE-2100 cell counter)	HIGH or LOW test result
Mean platelet volume (MPV) x Platelet count	FBC (XE-2100 cell counter)	LOW test result
Immature platelet fraction (IPF)	FBC (XE-2100 cell counter)	HIGH or LOW test result
Absolute reticulocytes (RETICS)	FBC (XE-2100 cell counter)	HIGH or LOW test result
Multiplate ADP test velocity	Multiplate	LOW test result
Multiplate ADP test amplitude	Multiplate	LOW test result
Multiplate AA test velocity	Multiplate	LOW test result
Multiplate AA test amplitude	Multiplate	LOW test result

Multiplate TRAP test velocity	Multiplate	LOW test result
Multiplate TRAP test amplitude	Multiplate	LOW test result
Multiplate ADR test velocity	Multiplate	LOW test result
Multiplate ADR test amplitude	Multiplate	LOW test result

2.6.3 Laboratory predictors: timings of test parameters

For each platform, test results have been classified as relevant to a 'rapid' or 'standard' time frame:

Standard Test result	**'Early phase' alternative**
Multiplate ADP test AUC	ADP test velocity
Multiplate AA test AUC	AA test velocity
Multiplate TRAP test AUC	TRAP test velocity
Multiplate ADR test AUC	ADR test velocity
ROTEM intem CT	*n/a*
ROTEM intem alpha	*n/a*
ROTEM intem mcf	Intem A5 or A10
ROTEM intem ml	*n/a*
ROTEM intem maxV	*n/a*
ROTEM intem maxV-t	*n/a*
ROTEM extem CT	*n/a*
ROTEM extem alpha	*n/a*
ROTEM extem mcf	Extem A5 or A10
ROTEM extem ml	*n/a*
ROTEM extem maxV	*n/a*
ROTEM extem maxV-t	*n/a*
ROTEM fibtem mcf	Fibtem A5 or A10
ROTEM extem mcf - fibtem mcf	Extem A5-Fibtem A5; Extem A10-Fibtem A10
ROTEM intem CT - heptem CT	*n/a*
TEG CK rmin	*n/a*
TEG CK angledeg	*n/a*
TEG CK ma	CK A30
TEG CK ly60	*n/a*
TEG CK rmin – CKH rmin	*n/a*

All other post-operative FBC, Multiplate, ROTEM and TEG test results are considered unlikely to predict CCB.

2.7 Other predictors and confounders

The role of other possible predictors and potential confounders will be investigated. These factors will be evaluated for association with the laboratory test results and association with the primary and secondary outcomes and will include age, sex, diabetes, type of surgery, operation urgency, pre-operative kidney function (estimated Glomerular Filtration Rate; eGFR) and pre-operative HCT. Post-operative platelet count will also be included in all models.

3. STUDY POPULATION

The study population is all patients aged 18 years or over attending Bristol Heart Institute for cardiac surgery between 30 March 2010 and 31 August 2012. All patients, with the exception of prisoners and those unable to give consent through mental capacity were eligible for the study.

3.1 Sample size

The study sample size detailed in the protocol (version 6, 21 March 2012) is 2400 which corresponds to the entire predicted patient throughput at Bristol Heart Institute for the 2 year study period, allowing for up to 10% of patients to opt-out.

Details regarding the sample size calculation and review are given in the SAP covering analyses of pre-operative test results.

3.2 Flow of participants

There is no study-specific follow-up for participants in the study, although clinical data extracted from the PATS database, which covers the period from admission for surgery until their date of discharge, will be used. Information collected prospectively on study subjects specifically for the study and recorded on case report forms (CRFs) relate to the period up until 12 hours after transfer to CICU.

The participant flow will be described via the flowchart (reference **Figure F1**).

3.3 Characteristics of non-study patients

In order to evaluate the representativeness of the study participants, demographic and clinical data recorded in PATS will be reviewed between the following groups:

1. Ineligible patients
2. Eligible patients who were not approached
3. Eligible patients who were approached but did not consent
4. Consented patients who did not provide a post-operative blood sample
5. Consented patients who did provide a post-operative blood sample where at least one laboratory test result was available

Classifications of reasons why eligible patients were not approached, and why eligible patients refused consent, or why samples were not taken from consenting patients will be documented.

Formal statistical comparisons of the final analysis group compared to those eligible and not included in the analyses will be undertaken using t-tests, Mann-Whitney tests and chi-squared tests as appropriate. Comparisons between all other groups will be descriptive.

3.4 Withdrawals

Patients (or clinicians on their behalf) can withdraw from the study at any time post-consent (including prior to their surgery). The data available for analysis will depend on the timing of the withdrawal (see section 3.6). Data collected up to the point of withdrawal will be retained for the analysis.

Data on all withdrawals is captured on the patient log CRF and will be tabulated (reference **Table T1**) with full details given in separate listings (reference **Table T2**).

3.5 Protocol deviations

The protocol states that all post-operative samples should be collected after the return of cell saver and/or pump blood, and after all "routine" protamine doses have been administered. All samples that are recorded as not being collected at this specific time will be reviewed and excluded if necessary. The overlap with the return of pump blood is of particular concern.

3.6 Analysis populations

The analysis population will be defined as all eligible, consenting patients who provided a post-operative blood sample. The final datasets will be restricted to those with laboratory assay results from the post-operative sample, *and* CRF data *and* clinical data extracted from PATS and INNOVIAN in order to define the primary outcome. Any consenting patients who had a post-operative sample collected but did not undergo surgery for medical or other reasons will be excluded from the analysis population. Comparisons between those in the final analysis population and those with CRF data and clinical data extracted from PATS and INNOVIAN in order to define the primary outcome, a pre-operative sample taken but incomplete laboratory assay results will be compared using standardised mean differences.

Involvement in any other UHBristol Clinical Trials and Evaluation Unit (CTEU) studies will be reviewed and any interventions deemed to have an impact will be reported. Details on how this information will be recorded are given in section 6.4.

4. COPTIC DATA COLLECTION

4.1 Data sources

Information will be collected from the following sources:

Data sources and identification details for linkage

Data type	Brief description	Identification for linkage
CRF Data	Information on time samples were taken, time of CICU admission, blood loss 6 and 12 hours post CICU admission, red blood cells (RBC) and non-RBC transfusions during operation and post-	STUDYID (identifies patient), COPTIC SAMPLEID (identifies sample), operation date, Hospital number/BRI Trust number

Data type	Brief description	Identification for linkage
	operatively up to CICU admission.	
Clinical data	Data extract from PATS Database including reoperation details (Section 5.1 (iii) and clinical outcomes as detailed in Section 2.5 (c).	Hospital number/BRI Trust number, name, sex, date of birth, operation date
Clinical data	Data extract from INNOVIAN, clinical information system of hourly data for time spent in CICU. These data include red blood cells (RBC) and non-RBC transfusions during CICU admission and clinical outcomes as detailed in Section 2.5 (c).	Hospital number/BRI Trust number, operation date
Clinical data	Creatinine test results for all consenting COPTIC patients, restricted to the closest test prior to the operation (pre-op) and any tests after the operation but within 7 days of the operation date (post-op)	BRI Trust number, date of birth
Laboratory test results	Results from all laboratory tests detailed in Section 2.5.1. These are transferred from the coagulation lab as a single file (FBC) or in the form of many separate files of weekly results (Multiplate, TEG and ROTEM).	All post-operative samples are identified by the COPTIC SAMPLEID (as in CRF data). The date of test will also be used to aid linkage.

4.2 Data linkage

Data from the sources detailed in the above Table will be individually matched in the following order, by the process outlined below:

Step 1 COPTIC CRF data will be linked with the clinical data as stored in PATS. Linkage will be achieved by matching against increasingly liberal matching criteria (i.e. relaxing the criteria required to accept a match), in the following order:

1. Matching on full name, date of birth and hospital number/BRI Trust number
2. Matching on full name and hospital number/BRI Trust number
3. Matching on full name and date of birth
4. Matching on hospital number/BRI Trust number, surname, date of birth (first name probable)
5. Matching on hospital number/BRI Trust number, first name, date of birth (surname probable)
6. Matching on hospital number/BRI Trust number and date of birth

The criteria that are the basis for each match (i.e. 1 to 6 above) will be recorded in the dataset. Records that do not match on operation date will be investigated, and excluded if differences are found to be correct. Instances where no matches can be found within PATS for COPTIC patients will be thoroughly scrutinised.

Step 2 All creatinine test results for tests completed between 27/3/2010 and 31/8/2012 provided by level 8, BRI, will be linked with the PATS and COPTIC file by Trust number and date of birth, and restricted to consenting COPTIC patients. Two separate datasets will be created:

- The sample relating to the closest time point prior to the operation date and time will be stored and referred to as the baseline creatinine measurement. If this is not available, the pre-operative creatinine value recorded in PATS will be used instead. This dataset will contain the pre-operative (baseline) creatinine measurement.
- The samples taken within 7 days of the operation date will be stored and formatted so these data are suitable for linkage back with the original dataset. This dataset will contain the post-operative creatinine measurements.

Step 3 Linked COPTIC CRF and PATS data will then be linked with the clinical data extracted from INNOVIAN. Linkage will be achieved by matching against increasingly liberal matching criteria (i.e. relaxing the criteria required to accept a match), in the following order:

1. Matching on operation date, hospital number/BRI Trust number
2. Matching on hospital number/BRI Trust number
3. Matching on operation date

The criteria that are the basis for each match (i.e. 1 to 3 above) will be recorded in the dataset. Records that only match on one common factor (2 and 3 above) will be investigated, and excluded if differences are found to be correct. Instances where no matches can be found within INNOVIAN for COPTIC patients will be thoroughly scrutinised.

Step 4 The linked dataset from Step 3 (CRF, PATS and INNOVIAN) will be linked with the creatinine datasets created in step 2.

Step 5 The linked dataset from Step 4 (CRF, PATS, INNOVIAN and creatinine data) will be linked with the FBC laboratory test results dataset, using the SAMPLEID variable. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked FBC result, or FBC results that do not link to COPTIC samples will be investigated thoroughly. Paper reports will be scrutinised to assist with correct values where duplicate values in the electronic files exist. Results arising from samples which were taken in error will be deleted. Any ambiguities or uncertainties arising during linking data through this step will consider findings and inconsistencies recorded in the Lab Deviation files.

Step 6 The linked dataset from Step 5 (CRF, PATS, INNOVIAN, creatinine and FBC results) will be linked with the Multiplate laboratory test results dataset, using the SAMPLEID variable. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked Multiplate result, or Multiplate results that do not link to COPTIC samples will be investigated thoroughly. Instances where only FBC or Multiplate results are available will also be reviewed. Paper reports will be scrutinised to assist with correct values where duplicate values in the electronic files exist. Results arising from samples which were taken in error will be deleted. Any ambiguities or uncertainties arising during linking data through this step will consider findings and inconsistencies recorded in the Lab Deviation files.

Step 7 The linked dataset from Step 6 (CRF, PATS, INNOVIAN, FBC and Multiplate results) will be linked with the ROTEM laboratory test results dataset, using the SAMPLEID variable. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked ROTEM result, or ROTEM results that do not link to COPTIC samples will be investigated thoroughly. Paper reports will be scrutinised to assist with correct values where duplicate values in the electronic files exist. Results arising from samples which were taken in error will be deleted. Any ambiguities or uncertainties arising during linking data through this step will consider findings and inconsistencies recorded in the Lab

Deviation files.

Step 8 The linked dataset from Step 7 (CRF, PATS, INNOVIAN, FBC, Multiplate and ROTEM results) will be linked with the TEG laboratory test results dataset, using the SAMPLEID variable. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked TEG result, or TEG results that do not link to COPTIC samples will be investigated thoroughly. Paper reports will be scrutinised to assist with correct values where duplicate values in the electronic files exist. Results arising from samples which were taken in error will be deleted. Any ambiguities or uncertainties arising during linking data through this step will consider findings and inconsistencies recorded in the Lab Deviation files.

In addition, data regarding the administration of anti-fibrinolytic drugs, recombinant human coagulation factors VII (rFVII) or fibrinogen concentrate will be provided by the Haematology department and checked against the data recorded in PATS. If there are any discrepancies, the data provided by the Haematology department will be used.

5. DEFINITIONS

5.1 Clinical concern about bleeding

The main objective of this prognostic study is to estimate how well test results from the post-operative blood samples predict CCB, i.e. bleeding that is likely to cause direct patient harm or indirect patient harm by precipitating a non-routine pro-haemostatic treatment or re-operation for bleeding for which no direct surgical cause is identified.

A participant will be classified as having experienced the primary outcome of CCB if any of the three circumstances below is documented within the designated time interval:

i. Post-operative blood loss (volume collected through chest drain) greater than 600mL at T5[1] (interval 2a).

ii. Intervention with a non-routine early pro-haemostatic treatment (EPT) defined as additional protamine after initial heparin reversal during interval 2b[2], fresh frozen plasma (FFP), cryoprecipitate, platelets, anti-fibrinolytic drug, recombinant human coagulation factors VII [rFVII] or fibrinogen concentrate during interval 2b (T3-T6 ; Figure 1).

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[1] Chest drain volume after 6 hours from admission to CICU is likely to be an unreliable marker of actual bleeding because of contamination with tissue fluid. There is no universally effective threshold for clinical concern about post-operative bleeding even at T5, so 600 ml total during this interval is a pragmatic clinical suggestion. Chest drain volumes at T6 will continue to be collected but it is very difficult to suggest a plausible 'threshold' value at this time point that will reflect 'actual bleeding'.

[2] Medical notes and details for those patients who only received additional doses of protamine and no other non-RBC products will be reviewed. Those with only one dose of protamine, administered after the post-operative sample is collected but prior to admission to CICU will be considered "routine" administration of protamine. The medical notes for all other instances will be reviewed and the protamine will be classified as "routine" or "therapeutic" as appropriate. Those considered to have received "routine" dose(s) of protamine will not be included in the definition of EPT.

iii. Reoperation for bleeding recorded during hospital stay and failure to demonstrate a surgical cause for bleeding [3].

5.2 Secondary endpoint definitions

i. Red cell transfusion expressed as: (i) any vs. none; (ii) total number of units transfused;(iii) >4 units vs. ≤4 units[4] occurring intra-operatively or during CICU stay (interval 2).

ii. Non-routine pro-haemostatic treatments (see section 5.1 for definition), occurring in interval 2, and expressed as: (i) any vs. none and (ii) total number of units or dose administered.

iii. Serious post-operative complications, i.e. death, myocardial infarction (MI), any stroke, acute kidney injury (AKI) and sepsis during hospital admission.

Secondary endpoints i) and ii) will be extracted from INNOVIAN and will cover the period of CICU admission, with information on therapeutic protamine for the time interval 2b extracted from the CRFs (time from the post-operative sample to 12 hours post-CICU admission). Secondary endpoints detailed in iii) will be extracted from the PATS Database or INNOVIAN. Due to known limitations in the completeness of the PATS database for some of these data, INNOVIAN data will also be utilised to identify these outcomes; firstly by the event being recorded in INNOVIAN and secondly by an electronic review of the following five free text fields extracted from INNOVIAN:

- Intensivists Review
- Problems (Physician Daily Review and Plan)
- Progress note (Physician Daily Review and Plan)
- Surgical Review
- CICU Ward round
- Plan of care on discharge

The search terms to be used are as follows:

	Innovian search terms	**Context terms**
Death	n/a (all captured in PATS/NHS Strategic Tracing Service [NSTS])	
Myocardial Infarction	Myocardial infarction Infarct	Q wave MI/myocardial infarction Non-Q wave MI/myocardial infarction

[3] In order to account for reoperations which were conducted due to surgical cause of bleeding, relevant electronic notes for all patients who required a reoperation will be reviewed by a cardiac surgeon (GJM). Any patient identification details will be masked. Those who, in the opinion of the surgeon, had a surgical cause of bleeding will be excluded from the definition of CCB.

[4] RBC transfusion of 1-2 units may occur at any time during or after surgery, usually in response to low haematocrit for which bleeding is only one cause. Thus, the number of red cell units transfused reflects poorly the extent of bleeding. However, larger volume RBC transfusions (arbitrarily defined as >4 units) more reliably reflect clinically important bleeding.

	MI STEMI NSTEMI ST elevation	Low cardiac output Cardiogenic shock IABP
Any stroke	Stroke Transient ischaemic attack, Hemiplegia Paralysis Paraplegia Dysphasia Cerebral/central nervous system (CNS) infarct/embolus	Loss of sensation CNS deficit Focal neurology Motor loss/deficit Sensation loss/deficit Brainstem Seizure Fit
Acute renal injury	Haemofiltration Haemodialysis Haemodiafiltration Dialysis Filtration Acute renal failure AKI Renal injury Kidney injury	New haemofiltration/dialysis CVVH RRT Already on dialysis
Sepsis	Chest infection Wound infection Mediastinitis Graft site infection Sternal wound infection Urinary tract infection Septic Sepsis Blood borne infection	Dehiscence Deep sternal wound Oozy sternum Positive blood culture Positive wound culture SIRS

Each of these secondary outcomes will be recorded as having occurred if there is evidence of a positive event within PATS or within INNOVIAN, including the review of free text.

5.3 Changes to the study objectives during the course of the study

These details differ slightly from those in the protocol (version 6, 21 March 2012):

- In the protocol, all outcomes are defined as clinical outcomes, with no differentiation between primary and secondary outcomes.
- The definition of CCB detailed in the protocol includes the statement that the intervention took place before carrying out a Thromboelastogram (TEG) analysis, or requesting a TEG analysis. This has now been removed from the definition of CCB at the agreement of the clinical investigators (GJM and AM).
- Clinical decision to return to theatre because of suspected bleeding has been added to the definition of CCB.
- The interval defined for red cell transfusion was defined in the protocol as during hospital stay; these data are available for CICU admission only. No details were recorded in the protocol in relation to the early pro-haemostatic treatments; these data are also available for CICU admission only.
- The serious post-operative complications detailed in the protocol (secondary outcomes iii) were "death, myocardial infarction, permanent stroke, renal failure (new requirement for dialysis) and sepsis". These have been amended to "death, myocardial infarction, **any stroke**, **acute kidney injury (AKI)** and sepsis". AKI will be defined using a modified KDIGO AKI Work Group laboratory definition[5]; AKI is present if serum creatinine increases by ≥26.5 μmol/l from the pre-operative value within 48 hours of surgery OR serum creatinine increases ≥1.5 times the pre-operative value within 7 days of surgery. The decision to replace "renal failure (new requirement for dialysis)" with AKI using creatinine data was made in the light of the amount of missing information in PATS.

6. DERIVATIONS

6.1 Primary endpoint

The rules and data sources for the primary endpoint of CCB are as follows:

New variable	Rules	Data sources
CCB (binary)	If post-operative blood loss 6hrs post CICU admission > 600mL OR intervention with a pro-haemostatic treatment (see section 5.1 for the definition) during interval 2 [interval 1 or interval 2b for therapeutic protamine] OR reoperation for bleeding where no surgical cause for bleeding is identified.	CRFs (blood loss data) PATS (return to theatre due to suspected bleeding) INNOVIAN clinical information system and CRFs (transfusion data) PATS (rFVII) Haematology Department (rFVII and fibrinogen concentrate)

6.2 Secondary endpoints

New variable	Rules	Data sources
RBC transfusion	Any RBC transfusion during interval 2	INNOVIAN clinical information

[5] Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Kellum *et al*. Critical Care 2013, 17:204

New variable	Rules	Data sources
(binary)	(the time between post-operative samples are taken and discharge from CICU).	system
RBC >4 units (binary)	Any RBC transfusion >4 units during interval 2 (the time between post-operative samples are taken and discharge from CICU).	INNOVIAN clinical information system
Non-routine pro-haemostatic treatments (binary)	Any platelet, FFP, cryoprecipitate or rFVII transfusion during intervals 1 and 2 (the time between post-operative samples are taken and discharge from CICU), or therapeutic protamine during interval 2b (the time between post-operative samples are taken and 12 hours post CICU-admission).	INNOVIAN clinical information system and CRFs
Serious post-operative complications:		
Death (binary)	Vital status (alive vs. died in theatre OR died in hospital).	PATS Database (updated with NSTS data)
MI (binary)	Any MI occurring before discharge OR death from MI in hospital.	PATS Database, INNOVIAN clinical information system and death certificate data
Stroke (binary)	Any peri- OR post-operative neurological complication (stroke; transient ischaemic attack; hemiplegia; paralysis; paraplegia; dysphasia; cerebral/CNS infarct/embolus).	PATS Database, INNOVIAN clinical information system
AKI (binary)	Serum creatinine increased by ≥26.5 µmol/l from the pre-operative value within 48 hours of surgery OR serum creatinine increased ≥1.5 times the pre-operative value within 7 days of surgery.	Creatinine data
Infection during hospital admission (binary)	Any infective complication (chest infection; wound infection; mediastinitis; graft site infection; sternal wound infection; urinary tract infection; septic; sepsis; blood borne infection).	PATS Database and INNOVIAN clinical information system

6.3 Laboratory variables

More can be entered here

New variable	Rules
Coagulopathy	*To be decided*

6.4 Other variables

More can be entered here

New variable	Rules
Age	(Operation date – DOB)/365.25
BMI	Weight (kg) / Height (cm)^2 * 10,000
Time in theatre	Cumulative bypass time; cumulative cross-clamp time
Cardiac procedure	CABG, Valve, other (or combination)
Pre-operative kidney function	Pre-operative serum creatinine and dialysis information will be used to calculate estimated glomerular filtration rate (eGFR): Cockcroft-Gault formula: $eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$ Or, when serum creatinine is measured in µmol/L: $eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$ where Constant is 1.23 for men and 1.04 for women.
Blood products as units	Divide volume (ml) by 280 to obtain red cells units Divide volume (ml) by 225 to obtain platelet units Divide volume (ml) by 260 to obtain FFP units Divide volume (ml) by 175 to obtain cryoprecipitate units *Selected as averages of given NHSBT product specifications* NB: Calculation of total units will be defined as missing if the total doses=0 and any of intra-op, grey area, INNOVIAN data are missing. i.e. they will not be recorded as missing if any doses given at any of these times is recorded, even if data at least one other time are missing.
Operative priority	This will be defined as “elective” or “urgent”. Any few emergency or salvage procedures will be classified as urgent.
Cardiac procedure	This will be classified in four groups; CABG only; CABG+valve; Valve alone; other
Involvement in other studies	An indicator will be created for each trial as follows: a) (Y/N) for participation in HArVeST, PROMPT, VERDICT, Protection1 b) (n/liberal group/restrictive group) for TITRe2 c) (n/intervention group/control group) for PASPORT There will be no indicator for concurrent involvement in smaller trials.
Treatment group, time since withdrawal of dual anti-platelet therapy and cardiac procedure	These variables are to be combined. Any combination which included an “other” procedure was reviewed and classified according to whether or not the “other” procedure was considered to be the primary determinant of the risk of bleeding (from the point of view of the operation being done). Patients with an “other” procedure classified as being the determinant of bleeding became a new subset; all patients with an “other” procedure were reclassified according to their main procedure; for example “CABG and other” was reclassified as “CABG only.” If the only procedure was an “other” procedure judged to have a high bleeding risk, it was classified with the new “other” subset; if it was judged to have a low bleeding risk, e.g. ablation, the patient was excluded from the analysis population. The “other” procedures identified as having a high risk were K08 "Repair of double outlet ventricle

New variable	Rules
	(Clean)", K33 "Aortic root replacement" and L19 "Other replacement of aneurysmal segment of aorta". The final categories will be as follows: – CABG only: no treatment – CABG+valve: no treatment – Valve only: no treatment – CABG only: aspirin – CABG+valve: aspirin – Valve only: aspirin – ABG only: dual_anti-platelet 0-2 days – ABG only: dual_anti-platelet 3-5 days – ABG only: dual_anti-platelet 6-7 days – CABG+valve: dual anti-platelet – Valve only: dual anti-platelet – Other: high risk

7. STATISTICAL ANALYSES

Statistical analyses and reporting will be undertaken in accordance with the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [http://www.strobe-statement.org/index.php?id=strobe-home].

Initial laboratory parameters of interest are detailed in section 2.6, although further investigations into other laboratory parameters not detailed here may be undertaken if there is evidence of potential associations with the primary and secondary outcomes based on the findings from these parameters. Laboratory parameters of interest will be examined individually, and within models where results are adjusted for each other laboratory parameter.

The distributions of the laboratory parameters of interest (section 2.6) will be compared between those defined as having CCB but this was only identified through one of the criterion (section 5.1) with those who were defined as CCB identified through two or more of the criteria. The main objective of this part of COPTIC Study B is to estimate the strength of associations between laboratory test results from the post-operative blood samples and CCB. Analysis will focus on estimating these associations, with sub-group analyses undertaken to examine the relationships between the timing of withdrawal of anti-platelet therapy and post-operative platelet function tests and primary and secondary outcomes.

Further details are as follows:

A1: In all patients:

a) characterise the distributions of the measurements from each laboratory test result;

b) fit regression models to estimate associations between the results of laboratory tests and the primary clinical outcome, CCB (see section 5.1);

c) develop models that would be informative in predicting risk of CCB according to laboratory test results, both by consideration of these results as continuous

measures or as above or below thresholds to be defined using receiver operating characteristic (ROC) analysis.

A2: In the patient sub-groups *no anti-platelet drugs*, *aspirin alone* and *aspirin+ $P2Y_{12}$ blockers*:

a) characterise the distributions of the measurements from each laboratory test;

b) fit regression models to estimate associations between the results of laboratory tests and the primary clinical outcome, CCB (see section 5.1);

c) develop models that would be informative in predicting risk of CCB according to laboratory test results, both by consideration of these results as continuous measures or as above or below thresholds to be defined using receiver operating characteristic (ROC) analysis.

A3: In the patient sub-group *aspirin+ $P2Y_{12}$ blockers*:

a) characterise the distribution of time between withdrawal of $P2Y_{12}$ blocker prior to surgery and time of surgery;

b) fit regression models to estimate the association between the time of withdrawal of $P2Y_{12}$ blocker (either as a continuous or categorical variable) and the results of the primary laboratory tests (see section 2.6).

c) fit regression models to estimate the association between the duration of withdrawal of $P2Y_{12}$ blocker (either as a continuous or categorical variable) and CCB.

Initially the justification for the three separate subgroups would be sought by fitting models for the whole dataset and including interaction terms to formally test for differences between the groups.

The choice of fitting time of withdrawal as a continuous variable or as a category variable in b) and c) above will be based on the i) distribution of times (few discrete values or truly continuous), ii) the functional relationship between the time of withdrawal and the outcome (assessed using fractional polynomials) and iii) interpretability of the findings.

A4: In all patients:

a) fit separate regression models to estimate associations between the results of laboratory tests and the secondary outcomes (see section 5.2);

b) develop models that would be informative in predicting risk of each secondary outcome according to laboratory test results, both by consideration of these results as continuous measures or as above or below thresholds to be defined using receiver operating characteristic (ROC) analysis.

7.1 A priori expected relationships

For each patient characteristic, the following are likely to be associated with an INCREASED likelihood of CCB.

1. Increased age
2. Female
3. Diabetes yes
4. Other>CABG+valve>Valve>CABG

5. Urgent operative priority>elective
6. Decreased eGFR
7. Decreased preoperative HCT
8. Low preoperative platelet count
9. Aspirin+P2Y12 blocker (clopidogrel or prasugrel) >aspirin>no drugs
10. Decreased time from P2Y12 blocker withdrawal
11. Reduced BMI

7.2 Specific analysis procedure

The approach is to evaluate the separate platforms (Multiplate/preop; ROTEM; TEG; Multiplate/postop) individually in the first instance, and then evaluate whether the inclusion of additional platforms would improve this. The reason for considering groups of variables by platform (rather than simply choosing the 'best' subset of variables) is because there is no cost of using extra variables from one platform, but an extra cost of doing a second test. The specific steps are as follows:

Step 1

Review the prediction achieved by patient characteristics. Define an optimal model based only on "baseline" covariates[6]. There will be a separate model for

- **any CCB** (intra-operative or post-operative) and
- **post-op CCB** only

Comment: We expect these models to be consistent with one another, not least because the majority of events (post-op CCPB events) will be common to both models.

Step 2

Review of each test platform to obtain the best model for each, including the baseline covariates:

- pre-operative Multiplate in relation to **any CCB** (this model is interesting in its own right, but from a slightly different perspective, i.e. it allows the care team to make a plan to mitigate the risk of CCB or to prepare to manage it)
- pre-operative Multiplate in relation to **post-op CCB**
- post-operative Multiplate in relation to **post-op CCB**
- post-operative ROTEM in relation to **post-op CCB**
- post-operative TEG in relation to **post-op CCB**

*Comment: Each of these models will consider all variables for the test/timepoint, i.e. consider the test/timepoint as a "package." The first model stands alone for **any CCB**. For **post-op CCB**, this step will identify which of the latter four models is the best platform for predicting CCB, i.e. provides the best prediction. We hypothesise that the best platform will be one of the post-op platforms because the information from these is more 'proximal' to the outcome for these models, i.e. **post-op CCB**.*

For the best test/timepoint (assuming there is one best test), we will also investigate whether a model using test variables that are available early (for the test identified as best) performs as well as a model using the standard test variables available at "test completion."

[6] These are: age, sex, diabetic status (n/y), cardiac procedure (CABG only, CABG + valve, valve alone, other), operative priority (elective, urgent), eGFR, preoperative HCT, treatment group, time since withdrawal (anti-platelet group), body mass index (BMI), preoperative platelet function and participation in another clinical study.

Step 3	Test whether the addition of one of the other (post-operative) platforms improves this best model for **post-op CCB**. In practice, this is likely to be a test of whether Multiplate-post improves prediction compared to either ROTEM or TEG (or, adding ROTEM or TEG to Multiplate-post, since ROTEM and TEG are assessing similar mechanisms and Multiplate is assessing different ones). The combination of ROTEM and TEG together will not be considered. **For the test/timepoint** added at Step 3 (assuming that there is one), we will also investigate whether a model using test variables that are available early (for the test/timepoint added at Step 3) performs as well as a model using the standard test variables available at "test completion."
Step 4	Test whether the addition of the pre-operative Multiplate platform improves the model identified in Stage 3. **If Multiplate-pre** is added at Step 4, we will also investigate whether a model using test variables that are available early for Multiplate-pre performs as well as a model using the standard test variables available at "test completion."

7.3 Model fitting

Models will be fitted first for the standard time frame. Then, models will be fitted for the rapid time frame (see Table 2.6.3), testing whether this results in a "significant" deterioration in prediction. Comparing rapid and standard time frames is interesting because there may be a trade-off between potential benefits of earlier decision making (e.g. allows definitive treatment to be provided more quickly, thereby preventing blood loss) vs. potential harms (e.g. somewhat less good prediction).

The contribution of all of these baseline characteristics to the prediction of CCB were incorporated into a single score which was forced into models of laboratory predictors with a coefficient of 1.00 in order to enable comparisons between different models based on the various laboratory test platforms.

All laboratory measures from a platform were included for each model, with multivariable fractional polynomial techniques used to investigate the linearity of terms. All transformations and centering found to improve the fit of the models were implemented. Backward elimination was undertaken to select the final models with a threshold of inclusion of 0.05 and exclusion of 0.10. Initial models only considered the standard test results (see Table 2); subsequently the 'early phase' alternatives were considered for any terms that significantly contributed to the model.

The following models were constructed:

- **Model 2A:** pre-operative Multiplate in relation to **any CCB**
- **Model 2B:** pre-operative Multiplate in relation to **post-op CCB**
- **Model 2C:** post-operative Multiplate in relation to **post-op CCB**
- **Model 2D:** post-operative ROTEM in relation to **post-op CCB**
- **Model 2E:** post-operative TEG in relation to **post-op CCB**

Each model was then compared to a baseline model containing only the baseline characteristics in order to evaluate the contribution of the selected laboratory test results to the prediction of CCB. These are presented as receiver operating characteristic (ROC) curves.

7.4 Measures taken to avoid bias

In the COPTIC Study, the study population is as representative as possible of the reference cardiac surgery population due to consecutive patients who satisfy the pre-specified inclusion criteria being invited to take part and all who consent are recruited. Analyses have been described (see section 3.4) to characterise the subgroups of patients who were, and were not recruited. Laboratory analyses of coagulation factor and platelet function are performed in a laboratory that is separate from the site of clinical care of the study subjects and are performed for all patients recruited to the study. Laboratory test results are not made available to the clinicians responsible for direct patient care and would therefore not influence the clinical management of participants. All laboratory tests are performed in a blinded fashion, with the Biomedical Scientists being unaware of all clinical patient data that would identify whether they would be classified as having CCB.

As the post-operative samples are collected earlier in the working day, it is anticipated that laboratory analyses would be performed on the vast majority of samples.

Baseline (i.e. patient demography and past history) characteristics will be described for the eligible and consented patients and for the subcohort of patients available for analysis (**Tables T3** to **T4)**.

Continuous variables will be summarised using the mean and standard deviation (SD) (or median and inter quartile range (IQR) if the distribution is skewed), and categorical data will be summarised as a number and percentages (**Tables T5** to **T6**).

In order to assess the representativeness of the final analysis populations, the characteristics of those patients included in the analyses will be compared with those not included using data extracted from the PATS Database. All possible demographic factors will be considered. **Table T5** to **T6** represent what we expect to use to display differences and similarities between those included and those not included in analysis populations.

7.4.1 Descriptive analyses

Distributions of laboratory test results will be described graphically using histograms and density plots.

7.4.2 Adjustment in models

The intention is to adjust all models for potential confounders (see section 2.7)[7]. These will be identified as either by significantly contributing to multivariate models, with likelihood ratio p-value of <0.05, or by modifying the effect estimate by greater than 10%. Age and sex will be entered in all final models, regardless of individual statistical contribution.

Evidence of effect modification will be investigated, by fitting interaction terms into the statistical models and consideration of the effect on the fitted estimates.

7.4.3 Analysis models

All outcomes listed in the study protocol will be presented as per the template tables **Table T7** to **T8**. General methods of presentation are outlined below.

[7] i.e. factors associated with the both the laboratory test result(s) (exposure) and the outcome, unbalanced across the groups and not an intermediary step in the causal pathway from the exposure to outcome

The primary outcome (CCB) and secondary outcomes (any RBC transfusions> >4 units RBC transfusion, non-routine pro-haemostatic treatments, death, MI, any stroke, renal failure, AKI and infections during hospital admission) are all binary data.

These binary outcomes will be presented as numbers and percentages of patients. The association between laboratory test results and outcome will be quantified using logistic regression for binary data. Estimates will be presented as unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (95% CI). For these binary outcomes analyses will only be performed if more than ten patients in total experience the outcome.

The sensitivity and specificity of laboratory test results to predict the outcome will be described for different thresholds and laboratory tests will be compared using the area under the receiver operating characteristic (ROC) curve.

The association between time of withdrawal of $P2Y_{12}$ blocker and laboratory test results will be described using linear regression.

7.4.4 Statistical significance

For hypothesis tests and tests of interactions, two-tailed p-values<0.05 are considered statistically significant. Likelihood ratio tests will be used in preference to Wald tests for hypothesis testing.

7.4.5 Model assumptions

For all methods outlined, underlying assumptions will be checked using standard methods, e.g. residual plots. If assumptions are not valid then alternative methods of analysis will be sought. If outlying observations are found which cause models not to fit the data adequately, such observations will be excluded from the main analyses and comments made in footnotes. Sensitivity analyses may be performed to examine the effect on the study's conclusions of excluding outlying observations.

7.4.6 Sensitivity analyses

In order to aid understanding of the role of EPT within the definition of CCB, two post-hoc sensitivity analyses will be performed. This was decided in the light of a large proportion of patients classified as CCB having no excess bleeding (chest drain < 600 mL at 6 hours) and did not require a re-operation, but received pro-haemostatic treatments. Additionally, anecdotal evidence from consultant anaesthetists exists that a number of factors such as cardiac procedure are likely to affect their decision as to whether to administer these products.

The additional sensitivity analyses are as follows:

- Those defined as having received EPT but no observed chest drain > 600 mL by 6 hours post-operation, or re-operation for bleeding, will be excluded from the analyses and the models and ROC curves detailed in section 7 will be repeated.
- Those defined as having received EPT but no observed chest drain > 600 mL by 6 hours post-operation, or re-operation for bleeding, will be included in the analyses but reclassified as free from CCB.

7.4.7 Post-hoc sensitivity analyses

In recognition of the limitations of the EPT component of the primary outcome, a post-hoc approach was agreed where CCB would be defined as before, but those with those with

only 1 or 2 platelet transfusions, or with only 1 FFP transfusion would be excluded. Additionally those with >4 RBC units within 48hrs post-CICU admission would be included.

7.4.8 Missing data

In all tables missing data will be indicated by footnotes. If the amount of missing data differs substantially by outcome, the potential reasons will be explored.

Missing predictors or missing outcomes:

- If the proportion of missing data is less than 5% then complete case analysis will be performed (i.e. excluding cases with missing data).
- If the proportion of missing data is between 5% and 25%, marginal mean imputation will be performed. This involves imputing the most common category in the case of categorical data and the overall median or mean in the case of continuous data.
- If the proportion of missing data is above 25%, multiple imputation methods will be considered. A general imputation model that uses an iterative procedure to generate imputed values will be used to generate multiple complete data sets (e.g. using Stata's mi impute). The model of interest will be fitted to each of the complete data sets and effect estimates combined using Rubin's rules.

7.4.9 Limits of detection

Methods of handling laboratory test results that are below the limits of detection of the assay will be explored; these may be replaced with a value which is half the limit of detection, or fitted using a uniform distribution between a fixed lower value and the limit of detection.

7.4.10 Multiple testing

Formal adjustment may be necessary for multiple testing, such as adjustment for false discovery rates. Consideration will be taken in interpretation of results to reflect the number of statistical tests performed and the consistency, magnitude and direction of treatment estimates for different outcomes.

8. BIBLIOGRAPHY

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9. AMENDMENTS TO THE SAP

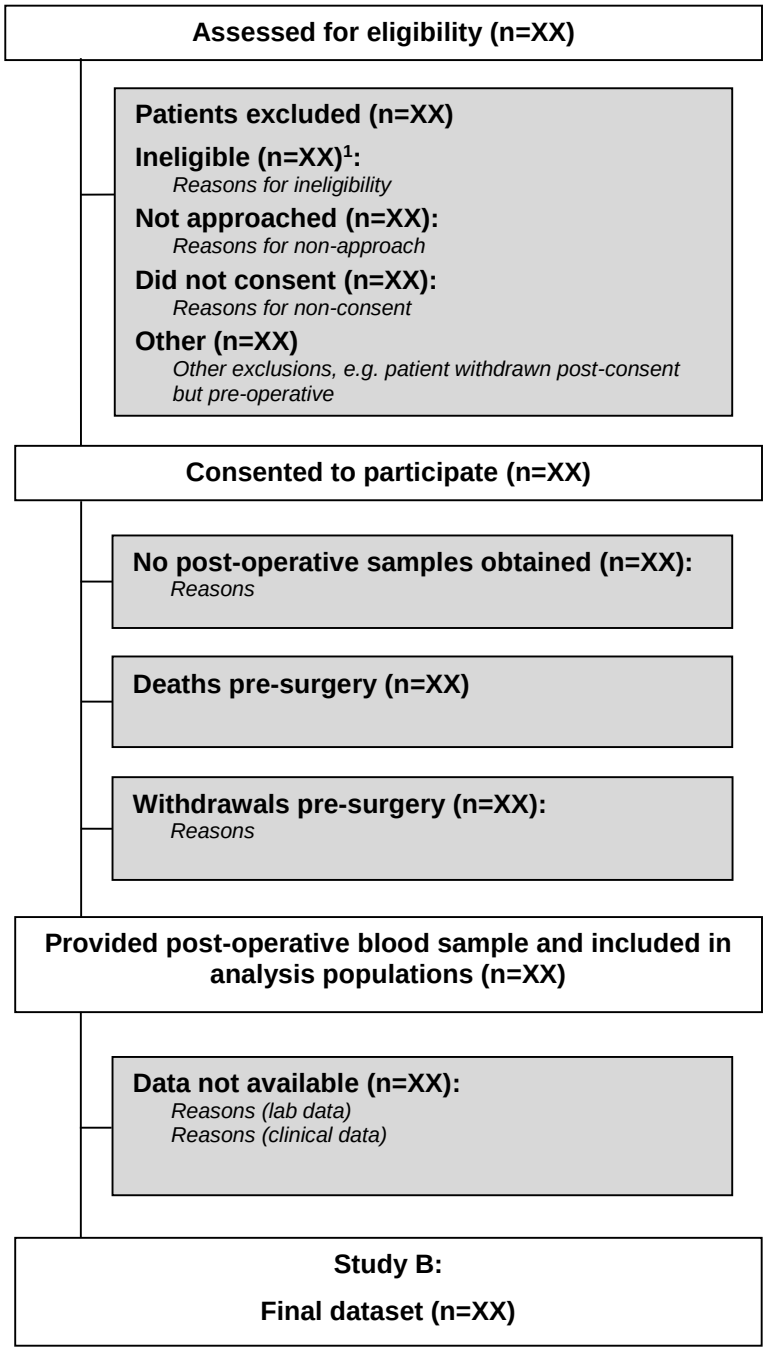
Previous version	Previous date	New version	New date	Brief summary of changes
1.0	14/10/2013	2.0		• Addition of TEG and ROTEM parameters. Version 1 detailed analyses pertaining to multiplate results only. • Addition of further details on transfer of blood volumes to doses using INNOVIAN data • Additional details on coding for operative priority and cardiac procedure • Additional information on the inclusion of data relating to other studies • Additional information on the analyses relating to secondary outcomes • Characteristics of non-study patients: definition of the final group changed to those eligible who provided a pre-operative blood sample where at least one laboratory test result was available • Clarification of the EPT collection period for the primary outcome, this is the first 12 hours of CICU admission time. • Addition of details on protocol violations where post-operative samples were collected prior to or during cell saver or pump blood return, or prior to routine protamine doses. • Addition of post-hoc sensitivity analyses to evaluate the role of EPT amongst those classified as having CCB
2.0	23/06/2014	3.0	09/09/2014	• Full details on the analysis approach defined in August 2014 by AM, BR and JH were incorporated. This approach pulls together the pre-

				operative and post-operative analyses so there is some overlap with the pre-operative SAP (version 4.0). This included the specific approach of analysis and definitions such as the categorization of patients by procedure, treatment and time since withdrawal. • References to subgroup analyses were removed as no longer relevant.
3.0	14/11/2014	4.0	24/09/2015	• Addition of the post-hoc analyses of a further sensitivity analysis based on CCB defined as before, but with a modified definition of EPT so that it excludes those with only 1 or 2 platelet transfusions, or with only 1 FFP transfusion and includes those with >4 RBC units.

APPENDIX A: SKELETON TABLES AND FIGURES

Section	Outputs	
Section 1 **Population**	**Tables, figures and listings detailing the study population**	
	Figure F1	Flow of participants
	Table T1	Withdrawals
	Table T2	Details of withdrawals
Section 2 **Baseline data**	**Summary tables of demographic information**	
	Table T3	Patient demography and past history
	Table T4	Intraoperative characteristics
	Table T5	Patient demography and past history: representativeness
	Table T6	Intraoperative characteristics: representativeness
Section 3 **Primary and secondary outcome data**	**Summary data and treatment estimates for primary and secondary outcomes**	
	Table T7	Primary outcome
	Table T8	Secondary outcomes

Figure F1 Flow of participants



Notes:
[1] *Some patients may be ineligible for more than one reason*