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

| | NAME | TITLE | SIGNATURE | DATE |
|---|---|---|---|---|
| **Author** | Jessica Harris | Medical Statistician | | |
| **Authoriser** | Chris Rogers | Co-head of CTEU | | |

| | |
|---|---|
| **Effective Date:** | 12/06/2015 |

**Table of contents**

## 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 |
| SYSMEX | Platform for reference tests |
| TEG | Thromboelastogram |
| TITRe2 | A multi-centre randomised controlled trial of Transfusion Indication Threshold |

| Acronym | Details |
|---|---|
| | Reduction on transfusion rates, morbidity and healthcare resource use following cardiac surgery |
| UHBristol | University Hospitals Bristol NHS Foundation Trust |
| VeRDiCT | Preoperative Volume Replacement vs. usual care in Diabetic patients having CABG surgery: a randomised controlled Trial |
| vWF | von Willebrand Factor |
| 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 reference test results** | Laboratory test results from SYSMEX, vWF or ETP |

# 1. INTRODUCTION

## 1.1 Scope

This statistical analysis plan (SAP) covers the analyses of the reference tests results from SYSMEX, vWF and ETP platforms for the post-operative blood samples collected for the COPTIC study (part of Study B). This SAP does not cover analyses of the rapid test results; these are documented in COPTIC pre-operative samples SAP [1] and COPTIC post-operative samples SAP [2]. 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.

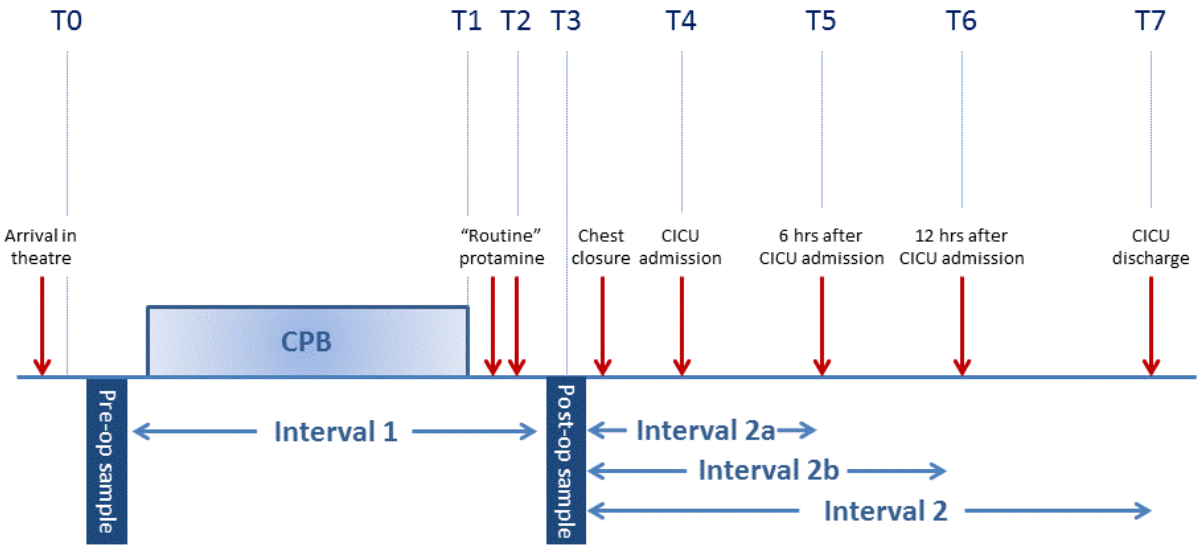
## 2.2 Overview of COPTIC study design

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 SYSMEX, vWF and ETP test results (part of Study B). A separate analysis plan has been written for the analyses of the other laboratory results from pre-operative and post-operative samples using the Multiplate, ROTEM and TEG platforms.

## 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 reference laboratory test or combination of tests of platelet function can be used to predict CCB. One aim of the COPTIC Study B is to estimate the association between the results from a panel of reference 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 reference 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 reference test results and other factors. The role of the reference test results will be considered separately in order to assess their association with the primary outcome.

The specific study objectives are as follows:

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

The reference test results from COPTIC Study B, have been sub-classified according to likelihood of an association with the primary outcome of CCB (objectives 1 and 2):

### 2.6.1 Primary reference laboratory predictors (test results considered likely to predict increased risk of CCB)

| Test result | Reference laboratory test 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 |
| Platelet count (PLT) | FBC (XE-2100 cell counter) | LOW test result |
| PT | SYSMEX | HIGH test result |

| | | |
|---|---|---|
| aPPT | SYSMEX | HIGH test result |
| Claus Fib | SYSMEX | LOW test result |
| anti-Xa | SYSMEX | HIGH test result |
| DDIC | SYSMEX | HIGH test result |
| FXIII | SYSMEX | LOW test result |
| vWF | vWF | LOW test result |
| ETP | ETP | LOW test result |

### 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 reference 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.

## 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 reference 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 reference 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 post-operative sample taken but incomplete reference 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-operatively up to CICU admission. | STUDYID (identifies patient), COPTIC SAMPLEID (identifies sample), operation date, Hospital number/BRI Trust number |
| 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 |
| Reference laboratory test results | Results from all reference 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 (SYSMEX, vWF and ETP). | All post-operative samples are identified by the COPTIC SAMPLEID (as in CRF data) or the coagulation number which can be linked to the COPTIC SAMPLEID by using the master list of samples. 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 SYSMEX laboratory test results dataset, using the SAMPLEID variable and/or coagulation number, via a separate linkage file that details both of these identifiers. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked SYSMEX result, or SYSMEX 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 7** The linked dataset from Step 6 (CRF, PATS, INNOVIAN, FBC and SYSMEX results) will be linked with the vWF laboratory test results dataset, using the coagulation number. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked vWF result, or vWF 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, SYSMEX and vWF results) will be linked with the ETP laboratory test results dataset, using the coagulation number. The date of test will be used to verify that the linkage was correctly undertaken. Any discrepancies between dates, or samples without a linked ETP result, or ETP 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 objectives of this prognostic study are to estimate how well reference 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).

[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'.

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).

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

Due to some non-routine EPT being administered peri-operatively, CCB can be defined at two timepoints; any CCB which occurs after the pre-operative sample is collected and post-operative CCB which occurs after the post-operative sample is collected.

### 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)

---

[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.

[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.

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

| | | |
|---|---|---|
| | Septic<br>Sepsis<br>Blood borne infection | |

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 $\mu$mol/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 | CRFs (blood loss data)<br>PATS (return to theatre due to |

[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 |
|---|---|---|
| | 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. | suspected bleeding)<br>INNOVIAN clinical information system and CRFs (transfusion data)<br>PATS (rFVII)<br>Haematology Department (rFVII and fibrinogen concentrate) |

## 6.2 Secondary endpoints

| New variable | Rules | Data sources |
|---|---|---|
| RBC transfusion (binary) | Any RBC transfusion during interval 2 (the time between post-operative samples are taken and discharge from CICU). | INNOVIAN clinical information 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 Other variables

| 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):<br>Cockcroft-Gault formula:<br>$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$<br>Or, when serum creatinine is measured in µmol/L:<br>$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$<br>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<br>Divide volume (ml) by 225 to obtain platelet units<br>Divide volume (ml) by 260 to obtain FFP units<br>Divide volume (ml) by 175 to obtain cryoprecipitate units<br>*Selected as averages of given NHSBT product specifications*<br>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:<br>a) (Y/N) for participation in HArVeST, PROMPT, VERDICT, Protection1<br>b) (n/liberal group/restrictive group) for TITRe2<br>c) (n/intervention group/control group) for PASPORT<br>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.<br>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 |

| New variable | Rules |
|---|---|
| | "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 (Clean)", K33 "Aortic root replacement" and L19 "Other replacement of aneurysmal segment of aorta".<br>The final categories will be as follows:<br>– CABG only: no treatment<br>– CABG+valve: no treatment<br>– Valve only: no treatment<br>– CABG only: aspirin<br>– CABG+valve: aspirin<br>– Valve only: aspirin<br>– CABG only: dual_anti-platelet 0-2 days<br>– CABG only: dual_anti-platelet 3-5 days<br>– CABG only: dual_anti-platelet 6-7 days<br>– CABG+valve: dual anti-platelet<br>– Valve only: dual anti-platelet<br>– 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 reference laboratory parameters of interest are detailed in section 2.6, although further investigations into other reference 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. Reference laboratory parameters of interest will be examined individually, and within models where results are adjusted for each other reference laboratory parameter.The distributions of the reference laboratory parameters of interest (section 2.6) will be compared between those defined as having CCB but 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 the reference 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) fit regression models to estimate associations between the reference laboratory test results and the primary clinical outcomes, any CCB and post-operative CCB (see section 5.1);

b) develop models that would be informative in predicting risk of any CCB and post-operative CCB according to reference 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) fit regression models to estimate associations between the reference laboratory test results and the primary clinical outcomes, any CCB and post-operative CCB (see section 5.1);

b) develop models that would be informative in predicting risk of any CCB and post-operative CCB according to reference 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 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 reference laboratory test results and the secondary outcomes (see section 5.2);

b) develop models that would be informative in predicting risk of each secondary outcome according to reference 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 and model fitting

The contribution of all of the baseline characteristics[6] to the prediction of CCB will be incorporated into a single score which will be forced into models of reference laboratory predictors with a coefficient of 1.00 in order to enable comparisons between different models based on the various reference laboratory test platforms.

Multivariable fractional polynomial techniques will be used to investigate the linearity of terms. All transformations and centering found to improve the fit of the models will be implemented. Backward elimination will be undertaken to select the final models with a threshold of inclusion of 0.05 and exclusion of 0.10.

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

## 7.3 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. Reference 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 reference 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 reference 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**).

[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.

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.

### 7.3.1 Descriptive analyses

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

### 7.3.2 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.

### 7.3.3 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.

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 reference 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 reference laboratory test results to predict the outcome will be described for different thresholds and reference 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 reference laboratory test results will be described using linear regression.

### 7.3.4 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.

### 7.3.5 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.

### 7.3.6 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

[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

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.

### 7.3.7 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.

### 7.3.8 Limits of detection

Methods of handling reference 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.

### 7.3.9 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

COPTIC pre-operative samples SAP and COPTIC post-operative samples SAP (saved in \Stats\Data Files\Studies\Coptic\Documents\SAP)

Kellum JA and Lameire N for the KDIGO AKI Guideline Work Group. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Critical Care 2013, 17:204.

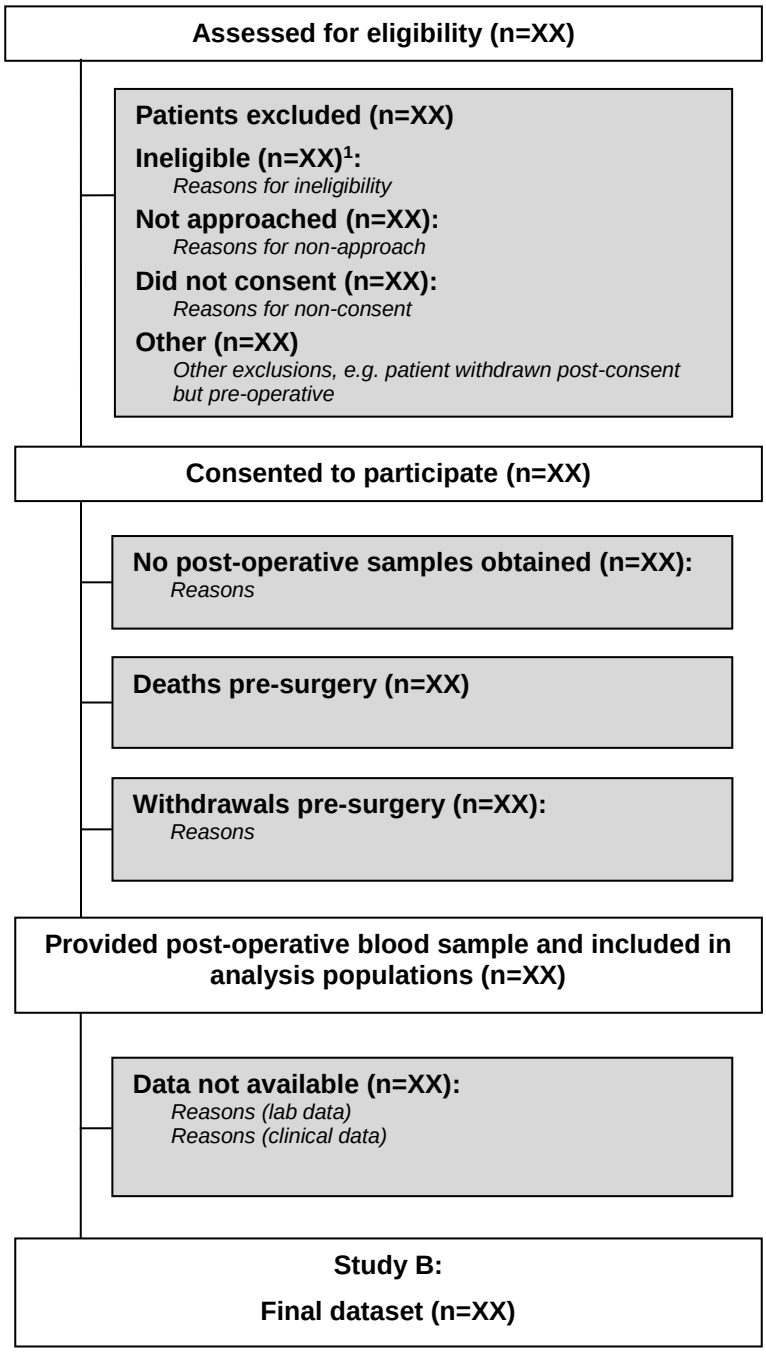
## 9. AMENDMENTS TO THE SAP

| Previous version | Previous date | New version | New date | Brief summary of changes |
|---|---|---|---|---|
| 1.0 | 12/06/2015 | 2.0 | 08/12/2015 | • Addition of FBC parameters as laboratory tests of interest.<br>• Addition of text to recognise that some analyses will be in relation to any CCB (recorded after the pre-operative sample was taken) and some will be in relation to post-operative CCB (recorded after the post-operative sample was taken) |
| | | | | • |

# 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*