Title of project

Coagulation and platelet laboratory testing in cardiac surgery

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Lay summary

Blood transfusion is a lifesaving treatment for excessive bleeding that is used commonly in patients having major surgery. However, blood transfusion is also expensive and has harmful effects on the immune system and circulation of recipients. As a consequence, the need to improve how blood is used was recognised as an NHS strategic priority in 2007.

Heart surgery often causes excessive bleeding and accounts for over 6% of all blood transfused in the UK. One important reason for this statistic is that heart surgery patients often have abnormal blood clotting. This can be because of tablet medication for heart disease taken before surgery or as an effect of the techniques used to perform heart surgery.

We will investigate whether it is useful to perform blood tests either just before, or just at the end of heart surgery to identify exactly how the blood clotting system is abnormal in each patient. The results of these tests could potentially lead to recommendations to give specific treatments to patients to improve blood clotting and reduce bleeding and blood transfusion.

We will perform this research over 24 months by taking two blood tests from 2400 consecutive patients having heart surgery at the University Hospitals Bristol. The blood will be analysed using specialist blood clotting tests so that we can identify exactly what abnormalities are present. We will test the blood using blood clotting analysers that are designed to be used in the operating theatre to find out how often these analysers correctly identify a clotting abnormalities and what additional test information is required to give the correct diagnosis when they do not. We will also investigate how our laboratory test results relate to the amount of bleeding and other complications in our patients. This information will show us the benefits of performing different kinds of tests so that we can recommend the best tests to perform in future.

1 Background

1.1 Research context

Blood transfusion is a lifesaving treatment for haemorrhagic shock. It is therefore used widely for patients undergoing surgery with high risk of bleeding. However, blood transfusion is an increasingly expensive resource and is associated with significant morbidity from immuno-modulation, infection transmission and pro-thrombotic effects. These limitations of blood transfusion are recognised in the Health Service Circular *Better Blood Transfusion* which promotes integration of evidence-based blood conservation strategies into clinical care pathways to minimise patient exposure to donor blood.[1] This initiative was recognised as an NHS strategic priority in 2007.[2]

Better Blood Transfusion is highly pertinent to cardiac surgery in which over 50% of patients currently receive blood transfusion.[3] About 40,000 CS procedures are performed anually in the UK and transfusion for cardiac surgery patients accounts for nearly 20% of all blood tranfused.[4] In cardiac surgery, blood transfusion is associated with the adverse outcomes of infection, myocardial infarction, renal failure and stroke and the associated indirect hospitalisation costs increase overall care costs by approximateley 10% per unit of blood transfused.[5] The clinical and resource burden to the NHS associated with blood transfusion is greatest in vulnerable patient groups such as women, the elderly and patients who require emergency surgery.

In order to generate evidence to support blood conservation strategies in cardiac surgery, blood transfusion practice has been identified as a major research theme in the Bristol Heart Institute. The National Institute for Health Research (NIHR) has funded an applied programme of research on this theme, entitled *Development of a multi-modality blood conservation strategy in cardiothoracic surgery*. This research programme includes complimentary randomised controlled trials and observational studies with the overall aims of identifying predictors of transfusion, reducing unnecessary use of blood and making blood transfusion safer. *Coagulation and platelet laboratory testing in cardiac surgery* is part of this programme. This project recognises that increased bleeding related to coagulopathy in cardiac surgery is a major cause of blood transfusion. It seeks to improve prediction and prevention of transfusion by improving the diagnosis and treatment of coagulopathy.

1.2 Project background

Cardiac surgery is associated with complex abnormalities in blood coagulation that are caused by patients' characteristics, drug treatments and the mechanical interventions that are necessary for surgery.[6] These abnormalities include pre-operative variables such as heritable coagulopathy and exposure to anti-platelet drugs. During cardiac surgery, coagulation factor defects may arise from dilution of blood, coagulation factor inactivation, heparin exposure and increased fibrinolysis during cardiopulmonary bypass (CPB). Abnormalities in platelet number and function may follow mechanical inactivation during CPB and from heparin exposure.[7] Intra-operative coagulation factor or platelet function defects in cardiac surgery are associated with increased bleeding, increased blood transfusion and with adverse clinical outcomes.[8-11]

Since both pre-operative and operative variables may contribute to coagulopathy and bleeding in cardiac surgery, we propose to study how the results of improved testing of coagulopathy (here, using reference laboratory tests) both before and after surgery can be used to benefit patients.

1.2.1 Pre-operative laboratory testing of coagulopathy

Mild heritable coagulation factor defects are prevalent in the general population and are associated with increased bleeding during surgery.[12] Cardiac surgery patients with coronary artery disease (CAD) may also be coagulopathic because of exposure to anti-platelet drugs. Although anti-platelet drugs are associated with bleeding in cardiac surgery,[10,13] they also have a strong cardio-protective effect in CAD which is greatest when two anti-platelet drugs are prescribed in combination.[14]

The importance of pre-operative exposure of anti-platelet drugs is recognised in current international guidelines which suggest temporary withdrawal of anti-platelet drugs before surgery.[6,15] These guidelines specify that for patients receiving dual therapy with clopidogrel and aspirin, clopidogrel only should be withdrawn 5-7 days before urgent cardiac surgery and all anti-platelet drugs should be withdrawn at least 7 days before elective cardiac surgery.[6,15] This approach aims to reduce surgical bleeding whilst minimising loss of the cardio-protective effect of the anti-platelet drugs. The time-points identified in these guidelines are derived from experience of anti-platelet drugs in other surgical settings and the theoretical lifespan of a platelet, not on the basis of empirical comparisons of alternative cut-off time-points. The recommended cut-offs may not be optimal for cardiac surgery patients, even on average, and withdrawal of anti-platelet drugs at the same time-point for all cardiac surgery patients fails to recognise that there are individual differences between patients in the extent of platelet inhibition by anti-platelet drugs. For example, this guideline may be giving rise to increased bleeding in patients with poor platelet recovery and residual platelet dysfunction at surgery.[10]

Pre-operative heritable coagulopathy and the effects of anti-platelet drugs are readily detectable with reference laboratory assays and with some rapid throughput assay systems that are suitable for use in cardiac surgery theatres.[16] These assays potentially allow identification of individuals at greater risk of bleeding before cardiac surgery so that directed pro-haemostatic treatment can be given to reduce bleeding and transfusion.[15-17] Pre-operative laboratory testing of coagulation factor and platelet function has not yet been studied in large-scale clinical trials in cardiac surgery. However, the increasing clinical and economic pressures to minimise blood transfusion now demand that coagulopathy in this surgical setting is investigated in greater detail.

Justification

We will investigate the patient benefit of pre-operative laboratory testing of coagulation factor and platelet function in all cardiac surgery patients, who we will divide into two cohorts, i.e. patients receiving and not receiving dual-antiplatelet therapy while waiting for cardiac surgery.

Heritable coagulopathies have not previously been investigated in cardiac surgery patients and their impact on bleeding and outcome following surgery are currently unknown. Therefore, in patients not receiving dual antiplatelet therapy, the objectives will be to (a) estimate the proportion affected, (b) to classify the heritable coagulopathies and (c) to quantify the risk of bleeding in affected patients. We will also model the potential economic benefits of preoperative testing, although we recognise that heritable coagulopathies are likely to be rare in this cohort and that, consequently, preoperative testing may not be cost-effective.

In patients receiving dual antiplatelet therapy, we have previously shown that the subset of urgent cardiac surgery patients who do not undergo withdrawal of dual anti-platelet therapy before surgery subsequently require more blood transfusion and have poorer clinical outcome than patients undergoing elective cardiac surgery.[5] This scenario affects approximately 7000 cardiac surgery patients annually in the UK. Since the potential patient benefits of pre-operative laboratory testing are greatest in this group, we will evaluate in detail the current practice of clopidogrel withdrawal in these patients. Currently, clinical constraints impose variation in the timing of clopidogrel withdrawal and we will exploit this variation to determine the relationship between laboratory markers of platelet function

and bleeding/ transfusion. Our study will: (i) describe individual differences between subjects; (ii) assess whether the current recommended cut-off falls at an appropriate percentile in the distribution (on average); (iii) quantify the risk from late withdrawal, and (iv) estimate the clinical benefit from instituting a patient-specific approach to management. We will also model the potential economic benefits of either modifying the existing guideline or of introducing platelet function testing in all, or selected cardiac surgery patients. If platelet function or coagulation factor testing is beneficial, our study will define the format and technical specification necessary for effective rapid-throughput testing devices in this setting.

1.2.2 Post-operative laboratory testing of coagulopathy

In comparison to pre-operative laboratory testing, the patient benefits associated with testing coagulation factor and platelet function after cardiac surgery are better established and this practice is already part of standard clinical care. In view of the dynamic nature of coagulopathy during cardiac surgery, rapid-turnaround visco-elastic haemostasis assays systems, such as the thromboelastogram (TEG®) or Rotational thromboelastometry (ROTEM®), are used in the operating theatre as a 'point of care' (POC) test. TEG is commonly used in UK cardiac surgery centres but ROTEM® is being widely adopted in other specialties and countries.

The outputs from the TEG and ROTEM assays comprise simple numerical parameters that describe haemostatic function. These parameters are predictive of increased bleeding in cardiac surgery when the results are dichotomised using established thresholds.[17] The high clinical utility of TEG has encouraged widespread use of this assay internationally in cardiac surgery, usually in association with simple management algorithms that direct specific pro-haemostatic treatments according to simple criteria derived from the TEG assay outputs. One such management algorithm has been studied in detail in prospective randomised controlled trials and was observed to reduce bleeding and transfusion requirement compared to decisions based on clinician judgement alone.[18-19] This TEG-based algorithm is now the practice standard for management of coagulopathy in UK cardiac surgery centres and has guided treatment decisions in more than 2500 procedures at the Bristol Heart Institute.

The TEG and ROTEM assays are likely to have limitations for the treatment of coagulopathy in cardiac surgery. For the greatest patient benefit, laboratory investigation of coagulopathy in cardiac surgery must have high diagnostic accuracy in order to direct (from a management algorithm) the correct treatment decision for an individual patient. Increasing understanding of the mechanism of coagulopathy in cardiac surgery now raises significant concerns about the diagnostic accuracy of these assays in some circumstances. This is best illustrated by the TEG parameter *MA* which is affected by major abnormalities in platelet number or function.[20] Therefore, existing TEG management algorithms specify platelet transfusion for all patients with abnormal *MA*. However, the *MA* parameter is also affected by deficiencies in plasma fibrinogen and Factor XIII which are associated with bleeding in cardiac surgery and may require treatment with plasma infusion.[11] The *MA* parameter is also insensitive to platelet dysfunction from anti-platelet drugs,[17] which causes bleeding in CS but requires platelet transfusion. The *MA* parameter, therefore, specifies platelet transfusion inappropriately in some circumstances and fails to identify an appropriate need in others.

Justification

TEG has established patient benefit and remains an attractive POC assay system in cardiac surgery . However, improving the diagnostic accuracy of TEG, or refining management algorithms directed by TEG results, offers the opportunity to improve current management and increase patient benefit further by reducing bleeding and transfusion. We hypothesise that ROTEM will have better diagnostic accuracy than TEG because it has greater specificity for some common coagulopathies in cardiac surgery such as fibrinogen deficiency. However, TEG and ROTEM have not been compared directly in cardiac surgery and there is no validated management algorithm for applying the findings of the

ROTEM assay in this setting. Our study will create a putative algorithm and determine whether it has better diagnostic accuracy than the TEG algorithm as currently used or following revision on the basis of the findings of the study.

The most likely way to improve diagnostic accuracy is to supplement the TEG or ROTEM assays with additional laboratory assays to identify coagulopathies which are not detected or which are misclassified by the TEG or ROTEM assays.[21] An array of novel POC assay systems are now available commercially which permit detailed investigation of specific coagulopathies in cardiac surgery,[22-24] and improving the diagnostic accuracy of coagulopathy testing by including additional POC tests is an attractive strategy. However, there is no high quality evidence about which additional assays should be performed and whether the potential increase in patient benefit from performing additional assays justifies testing.

In order to address this issue, we propose to evaluate the diagnostic impact of a range of laboratory reference tests of coagulopathy alongside current management of coagulopathy using TEG or ROTEM assays in a large cohort of patients having cardiac surgery. We will first evaluate the diagnostic accuracy of TEG or ROTEM assays in cardiac surgery against reference laboratory assays and then determine the relationship between diagnostic error and clinical outcomes that include bleeding. These data will then be used to generate alternative management algorithms that may, or may not incorporate additional POC assay systems. The potential clinical and economic benefits of these refined algorithms will then be estimated by modelling using the clinical and resource use data collected in the study combined with NHS costs obtained from reference sources.

2 Aims and objectives

2.1 Study aim

To estimate the patient benefit from measurement of coagulation factor and platelet function in patients undergoing cardiac surgery.

2.2 Study objectives

The patient benefit is likely to be greatest when laboratory testing of coagulopathy is performed either immediately before surgery, immediately after surgery or at both time points. It has already been established that there is some patient benefit from post-operative laboratory testing using TEG and this practice is now part of standard clinical care. Pre-operative laboratory testing is currently not performed routinely and the patient benefits of this practice are unknown.

In view of this difference in existing knowledge, the study designs and objectives for investigating pre-operative and post-operative laboratory testing are different. At the pre-operative time-point we will perform an observational cohort study to estimate the patient benefit from performing laboratory testing. A sub-group analysis will examine the relationships: (a) between the 'primary exposure' of timing of withdrawal of anti-platelet therapy and pre-operative reference platelet function tests and (b) between the 'primary exposure' of timing of withdrawal of anti-platelet therapy and clinical outcomes. At the post-operative time-point, we will perform a cross-sectional diagnostic accuracy study of the existing TEG-based laboratory testing practice and of ROTEM to establish whether there is additional patient benefit of modifying current practice.

The specific study objectives are as follows-

Pre-operative laboratory testing

A. In patients not on clopidogrel or prasugrel, use the laboratory assays to identify patients with pre-existing coagulopathies; estimate the prevalence of pre-existing coagulopathies; estimate associations between pre-operative reference laboratory reference assays of coagulation factor and platelet function and clinical concern about post-operative bleeding (CCB; see Study endpoints, 3.6).

B. For patients receiving dual anti-platelet therapy, estimate the relationship between the time of stopping clopidogrel/prasrugrel treatment and laboratory markers of platelet function, with particular reference to departure from the 5-7 day practice standard time.

C. For patients receiving dual anti-platelet therapy, estimate the relationship between the time of stopping clopidogrel/prasrugrel treatment and CCB (see Study endpoints, 3.6), with particular reference to departure from the 5-7 day practice standard time.

Post-operative laboratory testing

D. Investigate empirical diagnoses of coagulopathy from all reference assay results; estimate the frequency of patients with each coagulopathy diagnosis and with a diagnosis of no coagulopathy.

E. Compare empirical and clinical diagnoses (based on clinical actions) of coagulopathy.

F. Establish cut-off criteria for reference assays, TEG and ROTEM parameters to optimise their ability to predict empirical diagnoses; compare the diagnostic accuracy of the TEG and ROTEM parameters.

Common objectives

G. Model the potential patient benefits and resource use consequences of introducing pre-operative laboratory testing and of modifying current post-operative laboratory testing protocols to achieve higher diagnostic accuracy.

H. Determine the format and minimum technical specification necessary for an effective pre-operative and post-operative assay systems to achieve maximum patient benefit.

3 Plan of Investigation

3.1 Study design

Prospective, single-centre observational study of coagulation and platelet function determined from venous blood samples obtained at preoperative and post-operative time-points

3.2 Study population

3.2.1 Inclusion criterion

i. Age >18 years undergoing cardiac surgery at Bristol Heart Institute

3.2.2 Exclusion criteria

i. Prisoners
ii. Patients unable to give prospective or retrospective consent through mental incapacity

3.3 Research procedures

3.3.1 Clinical management of study subjects

Participants will undergo standard pre-operative, anaesthetic, surgical and post-operative care according to existing protocols.

For the research, two 22.5 ml blood samples will be obtained in the operating theatre at the following time-points:

i. Immediately before induction of anaesthesia
ii. Reversal of heparin anticoagulation

Blood samples will be taken from existing arterial lines that are inserted as part of standard clinical care. No additional venepunctures are required for this study. These samples represent a total additional blood requirement of 45 ml.

Decisions about intra- and post-operative haemostasis and transfusion treatment will be guided by TEG and other laboratory investigations, performed at the discretion of the responsible clinician in accordance with our routine institutional practice. These decisions will not be influenced by participation in this study.

3.3.2 Laboratory analysis

1. *Reference coagulation and platelet function assays*
    i. Prothrombin time
    ii. Activated partial thromboplastin time
    iii. Fibrinogen activity (Clauss assay)
    iv. Factor XIII activity (Berichrom assay)
    v. Heparin activity (anti-Xa assay)
    vi. Endogenous thrombin potential (Thrombin generation assay in platelet poor plasma)
    vii. von Willebrand factor activity (collagen binding assay)
    viii. D-dimer concentration
    ix. Platelet count
    x. Immature platelet count
    xi. Mean platelet volume
    xii. Maximum amplitude of platelet aggregation to ADP (Multiplate assay)
    xiii. Maximum amplitude of platelet aggregation to AA (Multiplate assay)
    xiv. Maximum amplitude of platelet aggregation to TRAP (Multiplate assay)

xv. Maximum amplitude of platelet aggregation to ADRENALINE (Multiplate assay)

2. *TEG analysis (post-operative sample only)*
   Estimates of R-time, K value, MA and LY30 parameters using kaolin and kaolin+ heparinase reagents.

3. *ROTEM analysis (post-operative sample only)*
   Estimates of R-time, K value and MA parameters using INTEM, FIBTEM, APTEM and HEPTEM reagents

3.3.3 Storage of clinical material

In order to enable retrospective analysis of confounding variables and for laboratory quality control and assay validation purposes, blood samples not used in the initial analyses will be stored at -80°C in a secure storage facility in the hospital laboratory for the duration of the study.

3.4 Patient recruitment

3.4.1 Study information and consent

The patient information sheet (PIS) and invitation letter will be posted to elective patients and faxed to those being transferred from other hospitals. Patients will be approached on admission and those that wish to participate will be asked to sign a written consent form for the study. In the rare event of a patient with capacity being unable to physically complete the consent form, verbal consent will be accepted and will be documented on the trial specific consent form and in the medical notes. The person taking consent and the patient (or someone of a qualifying relationship) will need to sign this request for participation.

After 6pm there is unlikely to be a member of the trials staff available to take consent. It is possible that an urgent patient may arrive after this time and the patient may, or may not, have had information regarding the study. For those who have not yet received information, it will be the clinician's responsibility to give the patient an overview of the study and to explain what participation would involve. Irrespective of whether the patient has had information in advance, verbal consent will be noted alongside procedural consent on the procedural consent form, signed by both the patient and clinician and a note written in the medical notes. Whilst all reasonable efforts will be made to return to the patient for written consent prior to surgery, in many cases this will only be possible after surgery. If a patient dies during/after surgery before giving written consent, but gave verbal prospective consent, continued participation will be assumed.

3.5 Duration of follow up

Duration of follow-up will be until discharge or death.

3.6 Definition study endpoints and primary exposure

3.6.1 Clinical outcomes

(a) Clinical concern about post-operative bleeding (CCB), i.e. intervention with a pro-haemostatic treatment (additional protamine after initial heparin reversal, FFP, cryoprecipitate, platelets, anti-fibrinolytic drug, rFVII or fibrinogen concentrate) before carrying out a TEG analysis, or requesting a TEG analysis. Note that this definition of CCB is independent of the results of a TEG analysis requested for clinical reasons.
(b) Post-operative blood loss at 6 and 12 hours.
(c) Red cell transfusion during hospital stay, expressed as (i) any vs. none and (ii) total number of units transfused.

(d) Operative plasma or platelet transfusion, expressed as (i) any vs. none and (ii) total number of units transfused.
(e) Serious post-operative complications, i.e. death, myocardial infarction, permanent stroke, renal failure (new requirement for dialysis) and sepsis.

3.6.2 Laboratory outcomes

Reference laboratory, TEG and ROTEM assay results recorded as continuous variables (see 3.3.2).

3.6.3 Primary predictor and outcome

The primary predictor and outcome of interest differ by objective. Therefore, they are defined below with respect to each objective separately.

A. Key predictors: results from pre-operative coagulopathy assays
Key outcome: clinical concern about bleeding.

B. Key predictor: time between stopping clopidogrel/prasrugrel medication prior to surgery and time of[surgery
Key outcomes: results from reference coagulopathy assays.

C. Key predictor: time between stopping clopidogrel/prasrugrel medication prior to surgery and time of surgery
Key outcome: clinical concern about bleeding.

D. Key predictors: results from post-operative reference coagulopathy assays
Key outcome: category of coagulopathy.

E. Key predictors: results from reference coagulopathy assays hypothesised to predict a category of coagulopathy (separate analyses for each category of coagulopathy)
Key outcome: category of coagulopathy (compared to category no coagulopathy).

F. Key predictors: results for parameters from TEG and ROTEM point-of-care analyzers hypothesised to predict a category of coagulopathy (separate analyses for each category of coagulopathy)
Key outcome: category of coagulopathy (compared to category no coagulopathy).

3.7 Measures taken to avoid bias

Selection bias will be minimised by selecting consecutive patients who satisfy the pre-specified inclusion criteria. Reference laboratory analyses of coagulation factor and platelet function will be performed in a laboratory that is separate from the site of clinical care of the study subjects and will be performed for all patients recruited to the study. Laboratory test results will be unavailable to the clinicians responsible for direct patient care and will therefore not influence the clinical management of participants.

3.8 Data collection

Almost all data will be collected from routine sources:

- Most data required to characterise (i) patients preoperatively, (ii) operations and (iii) post-operative complications will be collected through the audit infrastructure currently in place for all patients having cardiac surgery (Patient Administration and Tracking System, PATS).
- All reference assay data, and TEG and ROTEM data, will be maintained in the UHBristol electronic laboratory database.

- Data describing transfusions (RBC, platelets, fresh frozen plasma) will be obtained from the UHBristol blood bank.
- Data will be linked by pseudo-anonymised patient identifiers.

The data will be linked for analysis on the basis of a pseudonymised patient identifier, i.e. hospital number.

A minimal amount of data not available from the above sources (e.g. blood products in theatre given prior to sample collection and blood loss at 6 and 12 hours post admission to intensive care) will be collected from the medical records or post-operative care charts.

3.9 Sample size calculation

In view of the likely wide variation in laboratory markers of coagulation factor and platelet function at the two study time points and the presence of multiple independent variables that also affect bleeding and red cell transfusion requirement, it is necessary to evaluate a large study cohort to achieve the study objectives. We therefore propose a sample size of 2,400 which corresponds to the entire predicted patient throughput at our centre for the 2 year study period, allowing for up to 10% of patients to opt-out. (We expect the proportion of patients opting out to be much lower.)

For analysis of pre-operative laboratory testing, a sample size of 2400 will have >95% power to detect significant associations between continuous exposures and outcomes at a 2-tailed significance level of 0.01. The power of analyses with categorised exposure and dichotomous outcomes (e.g. objective B) will depend on the distribution of participants within categories and frequencies of outcomes. As a simple example, assuming that (i) the prevalence of clopidogrel withdrawal <4 days from surgery is 50% and (ii) the overall risk of excess bleeding is 15% (historical PATS data for Bristol), the study will have about 90% power to detect a relative risk of about 1.7 (11% vs. 19%) at a 2-tailed significance level of 0.05 in the subgroup of patients (approximately 800) taking clopidogrel prior to surgery.

For analysis of post-operative laboratory testing, a sample size of 2400 will allow sensitivity and specificity estimates to be determined with sufficient precision for clinical application. Precision depends on the proportion classified as ‘abnormal’ and the diagnostic accuracy estimate itself. For excess bleeding, again assumed to affect about 15% of patients, the sensitivity of test parameters will be estimated from a sample of about 300 (2000 x 0.15), with 95% confidence intervals ranging from about +/-6% for a sensitivity of 50% to +/-4% for a sensitivity of 90%. The proportion of patients classified as abnormal by different reference assays is unknown but, based on the above example, should be sufficiently precise to be clinically useful if platelet and coagulation pathway dysfunction is indeed associated with bleeding.

3.10 Planned recruitment rate

The study timeline is as follows

Months	Activity
0-3	Research approvals/set-up
4-28	Study subject recruitment and data collection
29-33	Data analysis
34-36	Dissemination

3.11 Participating centre(s)

This will be a single centre study at the Bristol Heart Institute and the University Hospitals of Bristol NHS Foundation Trust.

3.12 Likely rate of loss to follow-up

Duration of follow-up will be until discharge from hospital or death. Therefore, no loss to follow-up is expected.

3.13 Plan of analysis

As the primary predictors and outcomes differ, the analysis plan needs to be specified for each objective. The feasibility of some of the following analyses is dependent on the nature of the data. Preliminary descriptive analyses, but no regression modelling, will be carried out when half of the data have been collected. The analysis plan may be revised accordingly at this stage.

Pre-operative laboratory testing

A. In patients not receiving dual anti-platelet therapy (clopidogrel or prasugrel, and aspirin):
   a. characterise the distributions of the measurements from each assay;
   b. fit regression models to estimate associations between the results of reference assays and the primary clinical outcome, i.e. CCB bleeding (see 3.6);
   c. apply principal components or similar methods (depending on the nature of the distributions) to define and classify preoperative/heritable coagulopathies on the basis of the reference assay results;
   d. estimate the proportions of patients with different preoperative/heritable coagulopathies and no coagulopathy.

B. For patients receiving dual anti-platelet therapy:
   a. characterise the distribution of time between stopping clopidogrel/prasugrel medication prior to surgery and time of surgery;
   b. fit regression models to estimate the association between the time of stopping clopidogrel/prasugrel (either as a continuous or categorical variable) and the results of reference assays.

C. For patients receiving dual anti-platelet therapy:
   a. fit regression models to estimate the association between the time of stopping clopidogrel/prasugrel (either as a continuous or categorical variable) and CCAB.
   b. fit regression models to estimate associations between the results of reference assays and CCAB

Post-operative laboratory testing

D. In the entire cohort:
   a. characterise the distributions of the measurements from each assay;
   b. apply principal components or similar methods (depending on the nature of the distributions) to define and classify post-operative coagulopathies on the basis of the reference assay results;
   c. estimate the proportions of patients with different post-operative coagulopathies and no coagulopathy;
   d. compare the diagnostic accuracy of reference coagulopathy assays and TEG and ROTEM point-of-care parameters for diagnosing a category of coagulopathy (separate analyses for each category of coagulopathy).

E. In the entire cohort:
   a. use ROC analysis to identify optimal cut-off criteria for diagnosing a category of coagulopathy (separate analyses for each category of coagulopathy) from reference coagulopathy assays hypothesised to predict the category of coagulopathy.

F. In the entire cohort:
   a. use ROC analysis to identify optimal cut-off criteria for diagnosing a category of coagulopathy (separate analyses for each category of coagulopathy) from TEG and ROTEM point-of-care parameters hypothesised to predict the category of coagulopathy.

Common objectives

G. Model the potential patient benefits and resource use consequences of introducing pre-operative laboratory testing and of modifying current post-operative laboratory testing protocols to achieve higher diagnostic accuracy.
   a. In patients not receiving dual anti-platelet therapy, identify individual patients with preoperative/heritable coagulopathies diagnosed by reference tests; compare their outcomes and resource use with patients not diagnosed as having a preoperative/heritable coagulopathy.
   b. In patients receiving dual anti-platelet therapy, identify individual patients with significantly worse platelet function compared to the 'average' for a given time of stopping clopidogrel; estimate resource use as a function of time of stopping of clopidogrel (i.e. average impact of changing the recommended cut-off time of stopping clopidogrel); estimate resource use for the group of patients with significantly worse preoperative platelet function compared to the 'average' for a given time of stopping clopidogrel compared to other patients (i.e. impact of recommending the adoption of patient-specific cut-off times for stopping clopidogrel).
   c. In all patients, identify individual patients misclassified by optimal TEG and ROTEM test criteria (from E and F, above); compare their outcomes and resource use with patients not misclassified by optimal TEG and ROTEM test criteria.

H. Determine the format and minimum technical specification necessary for an effective pre-operative and post-operative assay systems to achieve maximum patient benefit.
   a. Fit regression models to estimate associations between the reference assays and CCAB; set optimal reference test cut-off criteria to identify patients classified as having (i) a preoperative/heritable coagulopathy or (ii) significantly poorer preoperative platelet function than average for a given time of stopping clopidogrel compared to other patients.
   b. Identify additional reference assays which, in addition to TEG or ROTEM parameters, minimise the misclassification arising from these point-of-care tests (Gc).

The proportion of patients correctly identified by diagnostic algorithms/models will be subject to 'optimism' bias. Ideally, this would be addressed by validating models on 'new' data (not used for developing the models), i.e. 'train-and-test'. The sample size available for this study is not large enough to permit this but alternative methods (cross-validation, boot-strapping, etc.) will be applied.

3.14 Frequency of analyses

A single analysis will be performed at the completion of the study

3.15 Economic issues

Health economic analyses will be undertaken by the Health Economics Research Centre (HREC) University of Oxford, a collaborating partner.

Models will be designed to estimate the costs and likely cost of the alternative approaches to preoperative and post-operative testing evaluated by the project. Specifically, the analyses will help to determine whether there are likely to be improvements in patient health outcomes (such as morbidity through complications) associated with implementing proposed (a) new and revised algorithms for making treatment decisions and (b) treatment interventions, and whether less use can be made of scarce NHS resources.

Data on health service resource use will be collected prospectively through PATS. These data will include the number and type of blood products and components administered complications, and hospital inpatient stay by ward type. Unit costs used to value this resource use will be obtained from credible sources including the National Blood Service and the Department of Health. Outcome measures will include serious complications following cardiac surgery, as previously described.

National estimates of the costs associated with transfusion will be inferred by scaling up the estimated costs using weights that appropriately reflect national characteristics. The current economic burden of transfusion in cardiac care has not previously been quantified, and so the analyses will provide valuable information for the NHS, as well as a robust baseline against which the potential national savings from better targeted therapy within this patient group can later be assessed.

4 Project management

4.1 Day-to-day management

This study is within the programme *Development of a multi-modality blood conservation strategy in cardiothoracic surgery* that will be managed by a dedicated programme manager and a dedicated clinical trial coordinator within the Bristol Heart Institute Clinical Trials and Evaluation Unit. These individuals will report to the principal investigator, who will be accountable to Chief Investigator and to the Cardiovascular Research Board of the University Hospitals of Bristol NHS Foundation Trust.

4.2 Steering committee and Data Monitoring and Safety Committee

The trial will be managed by an Executive group which will meet face-to-face approximately bi-monthly. The Executive group for the trial will be chaired by the Chief Investigator and will comprise the principal investigator, programme manager, the trial co-ordinator and co-applicants.

The project will be overseen by the Cardiovascular Research Board.

Cardiovascular Research Board membership

- Prof G Angelini (University of Bristol, Chair)
- Dr P Wilde (UHBristol, Clinical Director Specialised Services Division)
- Mr I Barrington (UHBristol, Specialised Services Divisional Manager)
- Dr J Sheffield (UHBristol, Trust Executive Lead for Research and Development)
- Mrs M Perkins (UHBristol, Research and Development Manager)
- Ms L Williams (UHBristol, Research and Development Manager)

- Mr B Morris (UHBristol, Specialised Services Divisional Finance Manager)
- Mr R Ascione (University of Bristol, Research Lead for Cardiac Services)
- Mr J Hutter (UHBristol, Clinical Governance Lead)
- Dr A Baumbach (UHBristol, Cardiology Consultant)
- Dr R Martin (UHBristol, Consultant in Congenital Heart Disease)
- Mr G Murphy (University of Bristol, Chief Investigator, NIHR Applied Programme)
- Prof B Reeves (UHBristol, CTEU, University of Bristol)

5 Ethical approval, consent, research governance and indemnity

5.1 Ethical review

UK NHS-REC approval for the study will be sought.

5.2 Consent

Arrangements for obtaining written informed are described in section 3.4.1. Consent for patients having elective and some urgent surgery will be requested prospectively (we estimate for >70%). Consent for patients having emergency or very urgent surgery will be requested retrospectively.

5.3 Data protection

Data will be collected and retained in accordance with the UK Data Protection Act 1998.

5.4 Data storage and sharing

5.4.1 Data storage

We will propose to the UK NHS REC that we retain all study documentation in a secure location during the study and for 5 years after the end of the study, when all patient identifiable paper records will be destroyed by confidential means. In compliance with the MRC Policy on Data Preservation, we will also propose that the fully anonymised dataset, a separate secure electronic 'key' with a unique patient identifier, and relevant 'meta'-data about the study be retained in electronic form indefinitely because of the potential for the raw data to be used subsequently for secondary research.

5.4.2 Data sharing

Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with the MRC Policy on Data Preservation and Sharing regarding scientific quality, ethical requirements and value for money. We propose that a minimum requirement with respect to scientific quality should be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review. The second file containing patient identifiers would be made available for record linkage or a similar purpose, subject to confirmation that the secondary research protocol has been approved by a UK REC or other similar, approved ethics review body.

5.5 Indemnity

This is an NHS sponsored research study as referred to in HSG (96)48 reference No.2. If there is negligent harm during the clinical study then the NHS body owes a duty of care to the person harmed. NHS indemnity covers NHS staff, medical academic staff with honorary contracts and those conducting the trial. NHS indemnity does not offer no-fault compensation and is unable to agree in

advance to pay compensation for non-negligent harm. Ex gratia payments may be considered in the case of a claim.

6 Dissemination of findings

The findings will be disseminated by presentation at international meetings, as well as by peer-reviewed publications and through patient organisations and newsletters to patients, where available. Since the study uses established clinical diagnostic assay device, there are no commercially exploitable findings from this study.

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