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TITRe 2

Title of project

A multi-centre randomised controlled trial of Transfusion Indication Threshold Reduction on transfusion rates, morbidity and healthcare resource use following cardiac surgery

Trial acronym: **TITRe 2**

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Summary in plain English

Indications for blood transfusion after cardiac surgery are poorly defined. Unnecessary blood transfusions increase healthcare costs both directly, because blood is an increasingly scarce and expensive resource, and indirectly, due to complications associated with transfusion. Transfusion may cause complications by reducing patients' ability to fight off infection and respond to the stress that surgery puts on the body, as well as (rarely) by transmitting viral infections present in donor blood. In the UK, cardiac surgery uses more than 6% of all donor blood (and about 10% of donor blood in the UHBristol, a tertiary cardiac surgery centre). Although the benefits of red cells for managing life-threatening bleeding are clear, the majority of decisions to transfuse after surgery are made on the basis of a patient's haemoglobin (Hb) level (a measure of the ability of the blood to transport oxygen around the body). The level that causes a doctor to transfuse a patient varies widely and randomised trials in non-cardiac surgical fields have shown that lowering the level that 'triggers' transfusion reduces complications as well as the use of blood.

The research will be carried out in a number of UK hospitals. Patients whose Hb level drops below the level at which transfusion is conventionally given will be assigned by chance to have decisions made: (a) more or less as they are now, or (b) only when the Hb level drops to a lower, 'restrictive' level. The primary outcome will be the number of infectious (sepsis) and ischaemic (stroke, heart attack or kidney failure) complications that occur during the first 3 months after surgery. We believe withholding transfusion until the lower Hb level is reached will reduce both complications and hospital costs.

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1. Background

Variation in red blood cell transfusion in cardiac surgery

Over 6% of all red blood cell usage in the UK occurs in cardiac surgery.[**2**] Although red blood cell (RBC) transfusion is essential in some cardiac surgical patients for the management of life-threatening haemorrhage, in most cases decisions to give a RBC transfusion are made because the haemoglobin concentration has fallen to a level or threshold at which the physician is uncomfortable.[**3**] The transfusion threshold varies from unit to unit and from surgeon to surgeon, contributing to the wide variation in blood usage observed in cardiac surgical units (25% to 95%).[**4**] The threshold variation stems from a lack of evidence as to what constitutes a safe level of anaemia following cardiac surgery.

Benefits, harms and costs of red blood cell transfusion

Viral, bacterial or prion infection, and haemolytic transfusion reactions are well publicised risks of red cell transfusion, but these are rare.[**5**] Immunosuppression, lung injury or organ dysfunction, on the other hand, may potentially occur in every recipient.[**6**] The risk of pneumonia has been observed to increase by 5% per unit of red cells or platelets.[**7**] In addition, retrospective studies investigating associations between RBC transfusion and specific morbidity after cardiac surgery have shown associations with nosocomial pneumonia,[**8**] sternal wound infection,[**9**] and severe sepsis.[**10**] A comparison of propensity matched pairs of transfused versus non-transfused patients, using data from over 3,000 patients treated in 145 European intensive care units, observed that RBC transfusion conferred a relative risk of mortality of 1.4 (95%CI 1.24-1.36).[**11**] RBC transfusion has also been reported to be associated with an increase in mortality up to five years after cardiac surgery.[**1,12**]

In addition to the direct costs of blood products themselves, morbidity associated with RBC transfusion increases hospital costs by prolonging ICU and hospital stay. In abdominal[**13**] and orthopaedic surgery,[**14**] avoiding RBC transfusion was associated with a reduction in total treatment costs of approximately $5,000 per patient. Findings of increased mortality up to five years after surgery suggest that there may be costs arising from long term transfusion-related morbidity or delayed complications.[**1,12**] There are also wider resource issues relating to the use of blood components nationally. Donor blood is an increasingly scarce resource, with up to 10% of donors excluded as a consequence of vCJD restrictions on the donor pool.[**5**] Increasing scarcity, as well as the introduction of measures aimed at increasing the safety of donated blood, is very likely to increase the direct costs of RBC transfusion.

Evidence about transfusion thresholds

There is little evidence about the optimal transfusion threshold for cardiac surgery patients. Healthy human subjects can tolerate haemoglobin (Hb) levels as low as 5 g/dL without adverse consequences,[**15**] and Hb levels as low as 7g/dL are safely tolerated in non cardiac surgery, trauma, and intensive care unit patients.[**16**] In the Canadian Transfusion Requirements in Critical Care (TRICC) study, non-cardiac ICU patients were randomised to either a restrictive (Hb level <7.0 g/dl) versus a liberal (Hb level <10.0 g/dl) transfusion trigger.[**17**] The restrictive trigger resulted in a 54% relative reduction in RBC transfusion and also a reduction in the frequency of organ dysfunction and 30-day mortality, effects which were attributed to a reduction in red cell transfusion associated morbidity. A subsequent meta-analysis of the TRICC and other studies confirmed that reducing RBC transfusion thresholds reduced postoperative transfusion rates; cardiac complications showed a non-significant reduction.[**18**] The applicability of these observations to a cardiac surgery population are unclear because the level of anaemia considered to be 'safe' is thought to be higher in the presence of cardiac disease. However, a post hoc analysis of the subgroup of patients with coronary artery disease in the TRICC study found no difference in 30-day mortality between the restrictive and liberal threshold groups.[**19**] On the basis of the TRICC

study results, some cardiac units in the UK routinely use a transfusion trigger of 7g/dL without any apparent detriment to patients.[**20,21**] However, to date, there has been no high quality randomised trial of different post-operative RBC transfusion thresholds in a UK population of cardiac surgery patients. A Brazilian randomised trial recently found no difference between liberal and restrictive transfusion thresholds (testing a hypothesis of non-inferiority).**[22]** The Data Monitoring and Ethics Committee has considered the design and features of this trial, and its findings, and has recommended that the TITRe2 trial should continue.

Summary of existing evidence

There has been no high quality randomised controlled trial (RCT) of alternative RBC transfusion thresholds in patients having cardiac surgery to date (other than the pilot study for this trial, which was greatly underpowered). It is important to investigate this question in patients having cardiac surgery because, compared to other patient populations, these patients are at greater risk of myocardial ischaemia due to coronary artery disease, and systemic tissue hypoxia in the presence of severe anaemia due to impaired cardiac output. In addition, cardiac surgery is a specialty which uses a large amount of blood. Increasing recognition of the risks of RBC transfusion,[**23**] coupled with the increasing costs of this potentially diminishing resource have led to calls for good quality prospective randomised trials to determine the relative risks and benefits of anaemia and RBC transfusion in this population.[**24**]

2. Aims and Objectives

The underlying hypothesis for the trial is that lowering the transfusion threshold for red cell transfusion from a haemoglobin (Hb) level of 9g/dL ("liberal", similar to current practice) to 7.5g/dL ("restrictive") will reduce postoperative morbidity and NHS costs.

Specific objectives of this multi-centre RCT are to:

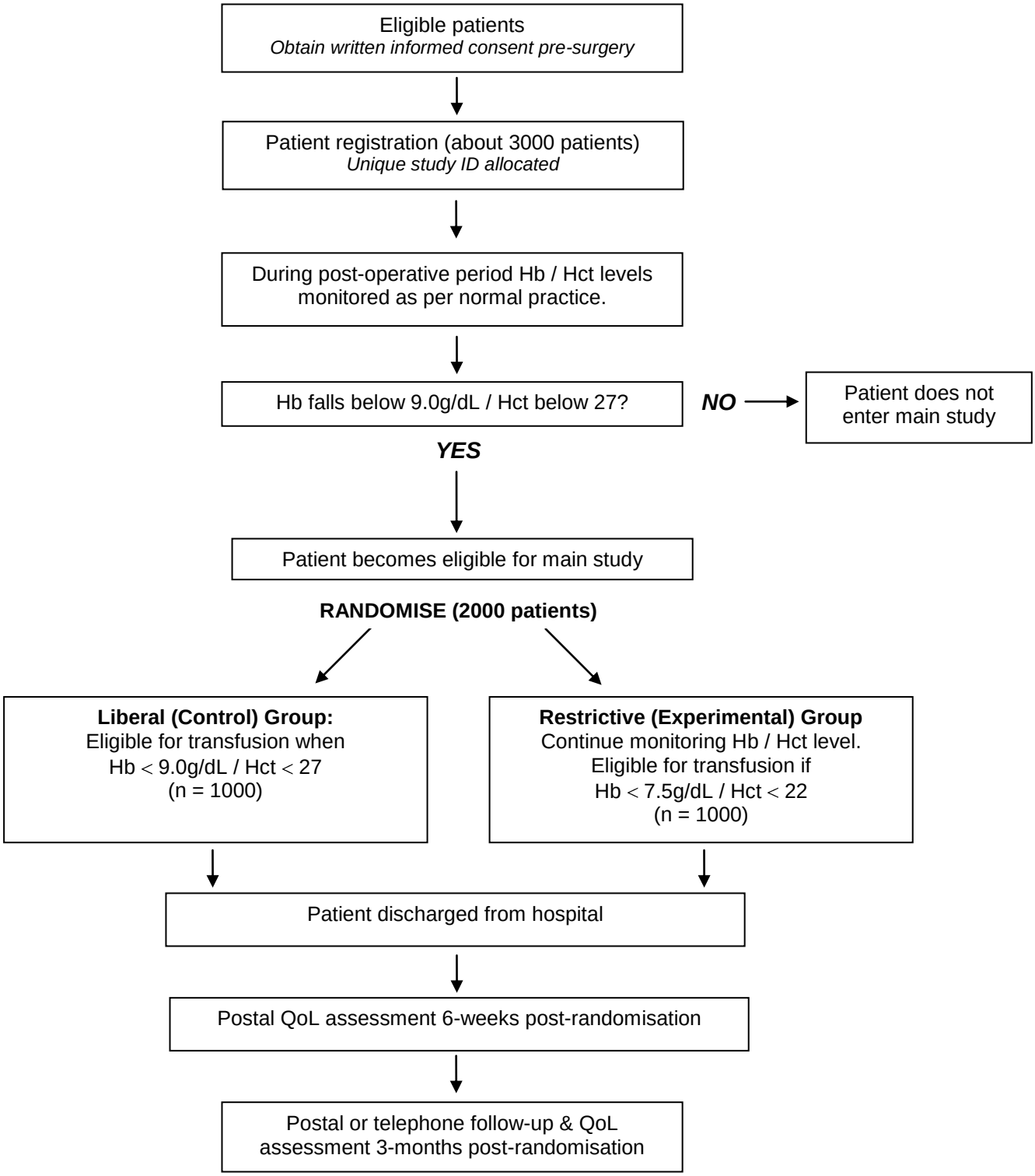
A. Estimate the difference in the risk of a post-operative infection or ischaemic event between restrictive and liberal transfusion thresholds.

B. Compare the effects of restrictive and liberal transfusion thresholds with respect to a range of secondary outcomes.

C. Estimate the cost-effectiveness of the restrictive compared to the liberal Hb transfusion threshold and describe this in terms of a cost-effectiveness acceptability curve.

3. Plan of Investigation

3.1. Trial Schema



3.2. Study design

The study will be a multi-centre, randomised controlled trial. The objectives (see section 2) will be addressed by randomising participants to either a restrictive or liberal threshold for RBC transfusion.

3.3. Description of intervention being investigated

The trial will compare two Hb thresholds for blood transfusion, 'liberal' and 'restrictive'. The thresholds are defined as follows:

1: Liberal (control, similar to current practice)
Participants randomised to this group will be eligible for transfusion if their post-operative Hb level falls below 9.0g/dL or haematocrit (Hct) falls below 27 at any time during their post-operative hospital stay on the cardiac intensive care unit (CICU) or cardiac surgical ward. One unit of RBC should be transfused and the Hb / Hct level checked before transfusing another unit. The objective should be to maintain the Hb level at or above 9.0g/dL or Hct at or above 27.

2: Restrictive (experimental)
Participants randomised to this group will be eligible for transfusion if their post-operative Hb level falls below 7.5g/dL or Hct falls below 22 at any time during their post-operative hospital stay on the CICU or cardiac surgery ward. One unit of RBC should be transfused and the Hb / Hct level checked before transfusing another unit. The objective should be to maintain the Hb level at or above 7.5g/dL or Hct at or above 22.

It is recognised that some cardiac intensive care units (CICUs) rely on Hct measurements from blood gas analysers when monitoring patients or that Hb may be measured less frequently than Hct. After a patient has been randomised to a group, if the Hb or Hct drops below the allocated threshold, a RBC transfusion should be given, even if there is a delay of up to 24 h from the most recent breach before the transfusion is prescribed. Where both Hb and Hct values are available at the same time, transfusion would be indicated if either of these values falls below the allocated threshold.

Clinicians will be allowed to transfuse, or refuse to transfuse, in contravention of the allocated threshold but must document the reason(s) why on the study case report form (CRF) (note: this does NOT constitute a patient withdrawal – see section 3.10).

Other aspects of post-operative care will be provided in accordance with local protocols. It is not practicable to insist that post-operative protocols are rigidly controlled. Stratification of randomisation within centres will ensure that variations in such protocols by centre do not introduce bias. Variations in protocols between centres are always likely to occur in the provision of usual care and, therefore, they can also be considered to enhance the applicability of the trial findings.

Haemoglobin thresholds for transfusion are controversial and different people will argue for different thresholds (in the same way that different clinicians will, in practice, transfuse at different thresholds). Our choice of thresholds takes into account the following considerations:

- The thresholds may appear close together but they span a 'densely populated' part of the distribution of nadir Hb (see **Figure 1**);
- In cardiac intensive care, transfusing at Hb >9.0 g/dL is considered unacceptable by many clinicians, including some who work in centres that have expressed an interest in taking part;
- In cardiac intensive care, not transfusing until the Hb drops to below 7.0 g/dL is also considered unacceptable by some clinicians, including some at the lead site in Bristol

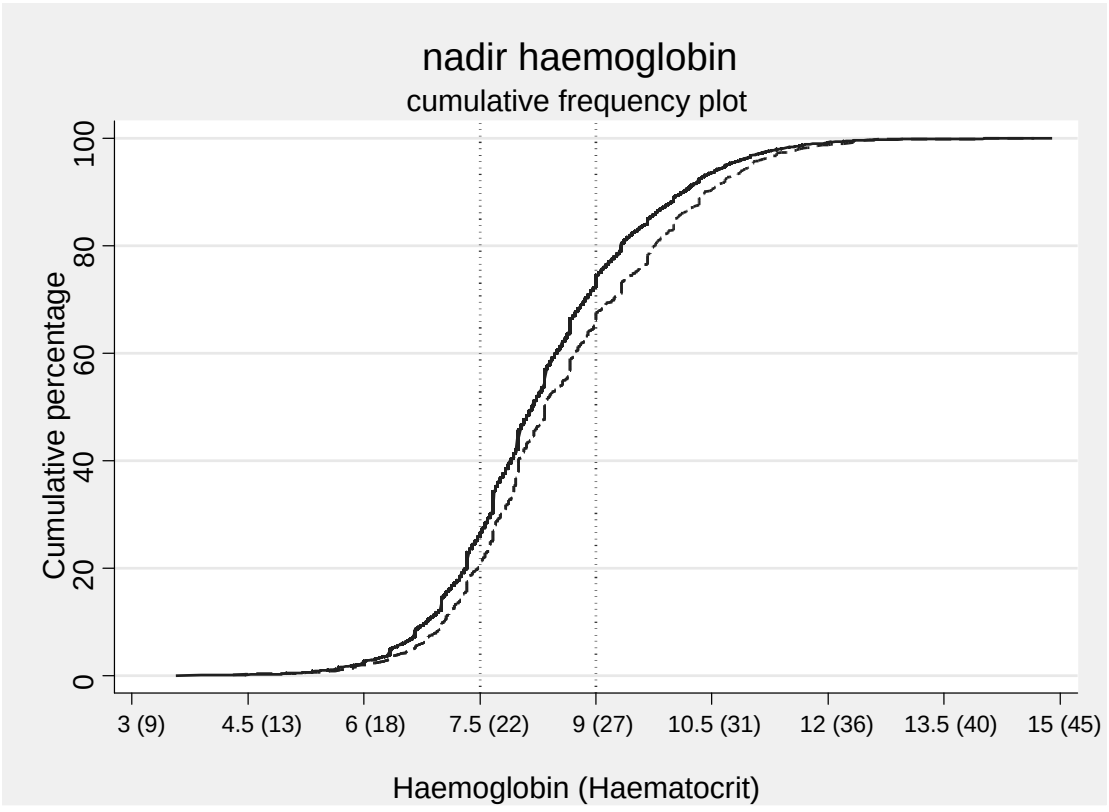
(one of the difficulties in the pilot trial) and in other centres that have expressed an interest in taking part.

Therefore, the current thresholds represent a compromise. Our proposed thresholds span the range of contemporary international practice, as experienced by researchers at the lead site who are surgeons and intensivists and have experience of transfusion practice in other European countries including Germany, Switzerland, and Italy. Among clinicians in interested centres, despite some unease at transfusing outside their existing protocols in some instances, there is a willingness to accept the proposed thresholds because of the perceived urgency of addressing the research question.

Patients who have consented to participate prior to surgery can become eligible for randomisation at any time during their post-operative stay on the CICU or cardiac surgical ward. Therefore, it is important that all clinicians caring for a consented patient must agree in principle to treat the patient according to the protocol. A decision can be taken by a clinician not to comply with the allocated transfusion threshold in individual cases, if thought to be in the patient's best interests. However, the reason for not complying must be documented at the time when the patient becomes eligible and / or the time at which transfusion is indicated according to the allocated protocol group.

Patients who have consented remain eligible for randomisation if their Hb falls below 9.0 g/dL / Hct below 27, irrespective of whether (a) a RBC transfusion has been given prior to randomisation, (b) a prior breach of the 9.0 g/dL Hb / Hct of 27 threshold was missed or (c) any element of the primary outcome has occurred.

Figure 1: Data for the distribution of nadir haemoglobin / haematocrit from recent observational analysis [1] both for the entire dataset (n=8,621, solid line) and for the most recent data (2003; n=1,106, dashed line). Vertical lines represent the restrictive and liberal protocols compared in this trial.



3.4. Study population

A restrictive transfusion threshold can, in principle, be applied to virtually all adult patients undergoing non-emergency elective cardiac surgery (this includes non-emergency cases admitted from home or non-emergency inpatient cases). We propose to use eligibility criteria that are as inclusive as possible to promote the applicability of the evidence obtained during the trial.

Inclusion criteria:

- Adults of either sex, aged ≥16 years undergoing cardiac surgery[1]
- Post-operative Hb level below 9.0g/dL or Hct below 27 at any stage during patient's post-operative hospital stay (i.e. on CICU or cardiac surgical ward)[2]
- Written informed consent

Exclusion criteria:

- Patients undergoing emergency cardiac surgery[3]
- Patients who are prevented from having blood and blood products according to a system of beliefs (e.g. Jehovah's Witnesses);
- Patients with congenital or acquired platelet, red cell or clotting disorders[4];
- Patients with ongoing or recurrent sepsis;
- Patients unable to give full informed consent for the study (e.g. learning or language difficulties);
- Patients with critical limb ischaemia[5]
- Patients already participating in another interventional research study

Details of all patients approached for the trial at each site, and reason(s) for non-participation (e.g. reason for being ineligible, patient or clinician preference or patient refusal) will be carefully documented.

[1] Cardiac surgery is defined as coronary artery bypass grafting, valvular or aortic surgery or surgical correction of congenital cardiac disease.
[2] No special investigations will be carried out to determine whether the post-operative Hb level has fallen below 9.0g/dL or Hct below 27; the trial will rely on investigations carried out at regular intervals (in CICU) or specially ordered by a doctor at other times as per standard local practice. Hb / Hct levels triggering randomisation can be obtained from laboratory measurements or blood gas analysers as per standard local practice. Over a 7 year period at the lead site in Bristol, 94% of all cardiac surgery patients who received a RBC transfusion had a nadir Hb <9.0g/dL.
[3] Emergency surgery is defined as surgery taking place before the end of the same working day as admission.
[4] Patients with iron deficient anaemia are not excluded
[5] Critical limb ischaemia is defined as rest pain in affected limb associated with peripheral vascular disease.

3.5. Method of allocation to groups

Participants will be randomly allocated to groups. Cohort minimisation will be used to achieve balance across the two arms of the trial; minimisation factors will be centre and operation type. Allocations will be generated by computer and concealed using an internet-based system (Sealed Envelope Ltd); this method has been used successfully in previous trials. Staff in participating centres will be able to gain limited access to the system using a password and pin. Information to identify a participant uniquely and to confirm eligibility must be entered before the system will assign a randomisation number and the randomised treatment allocation.

3.6. Patient recruitment

Potential trial participants will be identified from out-patient clinic lists (elective patients) and in-patient waiting lists (urgent patients). All potential participants will be sent or given a patient information sheet describing the study. Details of all patients approached for the study will be maintained by the centre on a screening log, and a unique study ID will be assigned on this log to all patients approached. The patient will have time to read the patient information sheet and to discuss their participation with others outside the research team (e.g. relatives or friends) if they wish. Most patients will have at least 24 hours to consider whether to participate or not although in some cases this time may be shorter (see section 7.7).

Patients will be seen in hospital by a member of the local research team (study clinician/research nurse/trial coordinator) who will answer any questions, confirm the patient's eligibility and obtain written informed consent. Once written consent has been obtained, the patient will be 'registered' into the trial by a member of the research team, who will enter the patient's details into the central study database (password controlled). The unique study ID number assigned to a patient on the screening log will be used throughout the patient's participation in the study (even though a randomisation number will also be allocated).

The Hb / Hct levels of consenting patients will be followed carefully during the post-operative period. If the Hb level drops below 9.0g/dL or Hct below 27, as determined from blood samples analysed as part of the patient's usual care, then the patient will become eligible for the main study and will be randomised at that time.

The person randomising the patient (clinical staff/research nurse/trial coordinator) will then seek to confirm the willingness of senior doctors looking after the patient to comply with the allocated transfusion protocol. If any doctor is unwilling, then the stated reason for not complying will be documented on the CRF. Randomisation will take place as soon as possible after the Hb level has dropped below 9.0g/dL / Hct below 27 and at most within 24 h of the breach occurring.

3.7. Duration of treatment period

The duration of intervention in the trial is the duration of the patient's care under the consultant cardiac surgeon ("finished consultant episode") or a maximum of 3 months after the date of randomisation, whichever is shorter. Almost always, the duration of care under the cardiac surgeon will be the period of hospitalisation after surgery. However, a few patients who develop serious complications, e.g. stroke, may be transferred to the care of another consultant in the same hospital, at which time the interventional period for the study will end.

3.8. Frequency and duration of follow up

The duration of follow-up in the trial is until the three month follow-up assessment questionnaires have been completed or until 3 months after randomisation if a participant refuses to complete the questionnaires. Participants will be posted a follow up questionnaire which will ask about surgical complications, adverse events and resource use during the

period from discharge until three months admission. If at the time of consent participants indicate they would prefer to be contacted by telephone, or if they do not respond to this questionnaire, they will be interviewed by telephone to ask for this information. The EuroQol EQ5D will also be posted to them to complete and return to the co-ordinating centre at six weeks and at three months after randomisation. Patients who are registered into the trial but not randomised will also be posted an EQ5D questionnaire three months after their operation (see section 3.13). Because RBC transfusion is associated with mortality in the longer term, we will follow up participants with respect to mortality using the UK population register, held by the NHS Information Centre. We propose to use the Medical Research Information Service (MRIS) for long-term flagging.

3.9. Definition of the end of the trial

This trial consists of two phases: an interventional phase and a three-month follow-up phase (see above). The end of the trial is defined as the final follow-up assessment (three-month postal questionnaire or phone call and the return of the EQ5D Questionnaire) for the final patient entered into the trial. If a patient is still in hospital or too ill to complete a follow up assessment at 3 months, their follow-up will be censored (except for passive follow-up through the UK population register).

3.10. Participants not managed in accordance with allocated protocol

Clinicians will be allowed to transfuse, or refuse to transfuse, in contravention of the allocated protocol group at any time, but must document the reason(s) why on the study CRF. This does NOT constitute a patient withdrawal.

If the consultant responsible for a participant decides it is in the best interests of a patient permanently to discontinue treatment according to the allocated protocol group, then the reason(s) for this, and the clinician taking this decision, must be documented on the CRF. Note: in this case the patient is NOT withdrawn from the trial (unless the patient withdraws consent, see below) and the patient should continue to be followed up in accordance with the protocol.

If a patient withdraws consent at any time then they should be withdrawn from the trial and this withdrawal must be recorded on the CRF.

3.11. Primary and secondary endpoints

Primary outcomes:

The primary outcome is a binary composite outcome of any serious infectious or ischaemic event in the first 3 months after randomisation. Note that randomisation will occur after surgery. The qualifying events listed in **Table 1** will be included, and verified in the manner described in the table.

Events occurring post-discharge will only contribute to the primary outcome if the potentially qualifying event resulted in admission to hospital or death. The exception to this is post-discharge wound infections, which will be ascertained using the ASEPSIS post-discharge surveillance assessment (see **Table 1**). Other suspected infectious events treated in the community will not be recorded because they cannot be validated and are less serious than peri-operative infections.

Events suspected to qualify for the primary outcome but not supported by objective evidence, e.g. test result of investigation report, that one or more of the definitions in **Table 1** has been satisfied, will be referred to an independent adjudication committee whose members will be blinded to random allocation. Research staff will collect as much evidence as possible about such events. The information will be presented to the Adjudication Committee for a final decision about whether or not the criteria for the primary outcome have been satisfied. The Adjudication Committee will consist of clinical specialists drawn from the Data Monitoring and

Ethics Committee (DMEC) supplemented by additional clinical experts in specialties not represented on the DMEC (e.g. neurology).

Death is not included as a component of the primary composite outcome because, if death occurs because of one of the included components, the component will precede death itself. Deaths that occur for other reasons are not hypothesised to increase because of RBC transfusion.

Table 1: Definition of serious infectious / ischaemic events for primary outcome

Infectious events	***Definition / method of verification***
Sepsis during index admission	Defined by the following two conditions, both of which must be satisfied for sepsis to be documented: (a) Antibiotic treatment for suspected infection, ***and*** (b) The presence of SIRS[6] within 24 hours prior to start of antibiotic treatment
Wound infection	ASEPSIS[**25**] score >20. Wounds will be assessed at least once during a participant's hospital stay and details of the ASEPSIS assessment added to the study CRF. A questionnaire will be posted for self-completion, or will be administered by telephone, at 3 months to identify wound infections arising after discharge.[**26**]
Ischaemic events	***Definition / method of verification***
Permanent stroke	Clinical report of brain imaging (CT or MRI), in association with new onset focal or generalised neurological deficit (defined as deficit in motor, sensory or co-ordination functions).
Myocardial infarction	Elevated post-operative peak serum Troponin I or T[7]
Acute kidney injury (AKI)	AKI Network criteria for AKI, stage 1, 2 or 3 (see below)[**27**] ***Stage 1:*** serum creatinine increase ≥0.3mg/dl (≥ 26.4μmol/l), OR >1.5 and ≤2-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR urine output <0.5ml/kg for 6 hours. ***Stage 2:*** >2 and ≤3-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR urine output <0.5ml/kg for >12 hours. ***Stage 3:*** >3-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR serum creatinine ≥4.0 mg/dl (≥354 μmol/l) with an acute increase of at least 0.5 mg/dl (44 μmol/l), OR urine output <0.3 ml/kg per hour for 24 hours or anuria for 12 hours, OR need for renal replacement therapy (RRT) irrespective of AKI stage at time of RRT.
Gut infarction	Laparotomy or post mortem

[6] SIRS - systemic inflammatory response syndrome. SIRS is central to the diagnosis of infective complications. It will be defined as ≥2 of the following conditions: temperature >38^{o}C or <36^{o}C; heart rate >90 beats/minute; respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg or <4.3 kPa; WBC count >12,000/mm^3 or <4,000/mm^3. Blood test results and temperature will be classified using standard reference ranges.

[7] Criterion levels of troponin I and T for defining a post-operative MI have not been established. Cut-off criteria will be set based on available blinded data before the database for the study is locked at the end of the study.

All available evidence suggests that RBC transfusion has a similar direction of effect for all components of the composite end point. Analyses of the Bristol database found that: respiratory and wound infections have similar frequencies; renal impairment is the most frequent ischaemic complication (about 4 times more common than myocardial infarction or stroke; **Table 2**).[**1**] (Note, however, that these complication frequencies were not based on the strict definitions described above.) **Table 2** makes two important points. First, with the exception of stroke (1%), the *overall* frequencies of components are similar (3-6%). Second, the direction of the effect of transfusion is the same for all components, albeit it is much less strong for myocardial infarction (MI). All of the above components are potentially life-threatening and we believe that the composite end point defined here avoids some of the common pitfalls in using a composite outcome.

We acknowledge that our decision to combine infection and ischemic outcomes (based on our previous findings**[1]**) has been taken partly to make the trial feasible (i.e. to yield a higher outcome frequency). However, recent publications by other researchers have described mechanisms for adverse effects of transfusion on MI, stroke and renal failure,**[28,29]** and unpublished data of our own shows differences in cytokine levels in transfused and non-transfused groups of patients.

Table 2: Frequency of components of the composite outcome in Bristol database[1]

	Composite infection outcome		**Composite ischaemic outcome**	
	Not transfused	**Transfused**	**Not transfused**	**Transfused**
Wound infection	2.1%	12.0%	-	-
Respiratory infection	2.6%	7.1%	-	-
Renal failure	-	-	2.8%	17.2%
Myocardial infarction	-	-	2.6%	3.1%
Stroke	-	-	0.3%	2.6%

Secondary outcomes:

Data will also be collected to characterise the following secondary outcomes at 3 months (unless otherwise stated):

(a) Units of red blood cells and other blood components transfused during a participant's hospital stay;
(b) Proportion of patients experiencing an infectious event;
(c) Proportion of patients experiencing an ischaemic event;
(d) EuroQol EQ5D;[**30**]
(e) Duration of intensive care unit (ICU) / high dependency unit (HDU) post-operative stay;
(f) Duration of post-operative hospital stay;
(g) All cause mortality.
(h) Cumulative resource use, cost, and cost-effectiveness.
(i) Significant pulmonary morbidity, comprising (i) initiation of non-invasive ventilation (e.g. continuous positive airway pressure ventilation), (ii) re-intubation/ventilation, or (iii) tracheostomy.

In addition, data will be collected for all patients to characterise compliance with the randomly assigned transfusion protocols. Hb / Hct are measured at regular intervals in CICU and the lowest Hb / Hct on each post-operative day will be collected (see below). When a transfusion decision is made for a randomised patient which is inconsistent with the allocated protocol (i.e. transfusion given even when the Hb / Hct has not reached the threshold allocated to a patient, or *vice versa*), the attending doctor will be required to explain the decision and this will be

documented on the CRF. In practice, research or ward staff will note when the 9.0 g/dL Hb / Hct of 27 threshold is breached for a patient who has consented, randomise the patient if consented, and then ask the attending doctor to confirm that the research protocol can be followed for the patient.

3.12. Measures taken to avoid bias

Concealed randomisation will prevent selection bias.

Every effort will be made to blind participants to their allocation. The success of participant blinding will be checked by asking participants if they knew what their allocation was at the time of their discharge from hospital and at 3 months (see 3.13).

It is not possible to blind clinicians and other NHS staff caring for patients to the random allocation of participants to restrictive or liberal threshold groups. Therefore, especial care is required in defining outcomes on the basis of objective criteria as far as possible, in order to minimise susceptibility to bias (see 3.11).

3.13. Data collection

Data collection will include the following elements:

1. Log of all non-emergency patients having cardiac surgery and those who are approached for the trial, including date when given the Patient Information Sheet;
2. Assessment of all patients who are approached against the eligibility criteria and, if ineligible, reasons for ineligibility;
3. Consent, and baseline information (e.g. operation type) required prior to randomisation (including responses to the EuroQol EQ5D), for all patients registered into the trial (whether or not they are randomised into the main study);
4. For randomised participants, the date and time when the Hb level falls below 9.0 g/dL or Hct below 27;
5. For all registered patients, post-operative data (collected at the time of discharge) including a summary of blood products received and other data to check compliance with protocol.
6. For all randomised participants, post-operative data collected during a participant's hospital stay will also include observations required for the primary outcome (e.g. temperature, heart rate, respiratory rate, results of haematology and biochemistry investigations), and secondary outcomes (e.g. duration of intensive / high dependency care, other key resource use (e.g. return to theatre, medications, units of blood components transfused), assessment of wounds for infection, see 3.11);
7. For randomised participants, data about whether a participant is blinded to random allocation on hospital discharge.

Anonymised data to characterise the patients who are approached about the trial (elements 1 and 2, above) will be recorded by research staff at participating centres on the trial Screening Log. After consent, research staff in participating centres will collect data on pre-printed CRFs and these data will be transferred promptly to a secure computerised database maintained on an NHS computer. Post-operative Hb / Hct levels in consented patients will be observed closely and any patients whose Hb level drops below 9.0g/dL / Hct below 27 will be randomised immediately, if possible, but randomisation may occur up to 24 h later. The threshold to which a participant has been randomised will be communicated to attending medical and nursing staff, and the date and time of randomisation recorded on the CRF; element 4, above). Data relating to operative details, postoperative morbidity, blood loss, haematological data and blood product usage will also be collected on the CRF (elements 5 and 6), including dates and times of relevant outcome events.

Three months after the operation, a questionnaire will be posted for self-completion, or will be administered by telephone (if a participant elects to be telephoned or fails to return the postal questionnaire), by staff at the co-ordinating centre (Clinical Trials and Evaluation Unit (CTEU), University of Bristol) and will include the following elements:

8. Questionnaire about surgical complications and other adverse events occurring after discharge (e.g. stroke, myocardial infarction); further details of any event suspected to contribute to the primary outcome (see section 3.11) or to meet the definition of a serious adverse event (see Section 6) will be sought, e.g. from the admitting hospital or the participant's general practitioner.
9. Questionnaire to identify surgical wound infections occurring after discharge (ASEPSIS post-discharge surveillance questionnaire).[**25**]
10. Health Economics / Resource Use Questionnaire.
11. Question asking whether a participant is aware of his/her random allocation.

Table 3: Schedule of Data Collection for TITRe2

	Pre-surgery	Day of surgery	When randomised	When in CICU/ward	At discharge	6-weeks after randomisation	3-months after randomisation
Eligibility	✓ [a]						
Written consent	✓ [a]						
Demographics and medical history	✓ [a]						
EQ5D Questionnaire	✓ [a]					✓ [b]	✓ [b]
Operative details		✓					
Hb/Hct level	✓ [a]	✓ [a]	✓	✓ [a]			
Summary of blood components transfused				✓ [a]			
Details of RBC transfusion		✓		✓			
Randomised allocation			✓				
Surgical complications & adverse events				✓			✓ [d]
ASEPSIS assessment of wound infection				✓ [c]			
Resource use data				✓			✓ [d]
ASEPSIS post-discharge surveillance							✓ [d]
Check participant blinded to allocation					✓		✓ [d]

a. Data collected for *all registered* participants (all other data to be collected for randomised participants only)
b. EQ5D at 6-weeks and 3-months post-randomisation assessed via postal questionnaire
c. Wounds will be assessed separately at least twice during a participant's hospital stay
d. Data collected via 3-month follow up postal questionnaires. This information may be obtained by telephone if the patient does not respond to the postal questionnaire or would prefer to be contacted by telephone

Finally, the EuroQol EQ5D will be posted to patients at 6-weeks post-randomisation and at 3 months after randomisation. Patients who are registered into the study but not randomised will also receive a postal EQ5D 3 months after their operation. If any questionnaire is not returned within the following 2-3 weeks, a reminder will be posted. If there is no response to the reminder, staff in the CTEU will telephone the participant to find out if there is a reason for non-response and to ask if they would prefer to complete the questionnaire over the phone. If a participant has indicated that he/she would prefer to be contacted by telephone, the CTEU will telephone the participant and complete the questionnaires over the phone.

3.14. Sample size calculation

The trial is designed to answer superiority questions.

The target difference in the primary outcome is based on data from observational analyses of the effects of RBC transfusion on the risk of the composite primary outcome (see 3.11). The estimated proportions of patients experiencing the primary outcome are 17% in the group allocated to the liberal threshold, and 11% in the group allocated to the restrictive threshold, i.e. a risk difference of 6%. A sample size of 1,468 is required to detect this difference with 90% power with 5% significance (2-sided test). For the sample size calculation, the estimated proportions of patients in whom any transfusion occurs in compliance with the allocated threshold (see **Figure 2**) are based on observational data.[**1**]

The estimated proportions of patients in restrictive and liberal groups experiencing the primary outcome are uncertain for several reasons:

- They are estimated from observational data, which are likely to be confounded to a greater or lesser extent;
- They are based on the RBC transfusion rate in Bristol for over 7 years (although transfusion rates in other centres are expected to be similar or higher based on anecdotal report);
- They are based on routinely collected data, using definitions for elements of the composite primary outcome which are not identical to those proposed for the trial;
- They are based on any vs. no RBC transfusion (i.e. the average number of units transfused), rather than on the number of units of RBCs likely to be transfused in patients who breach the liberal threshold.

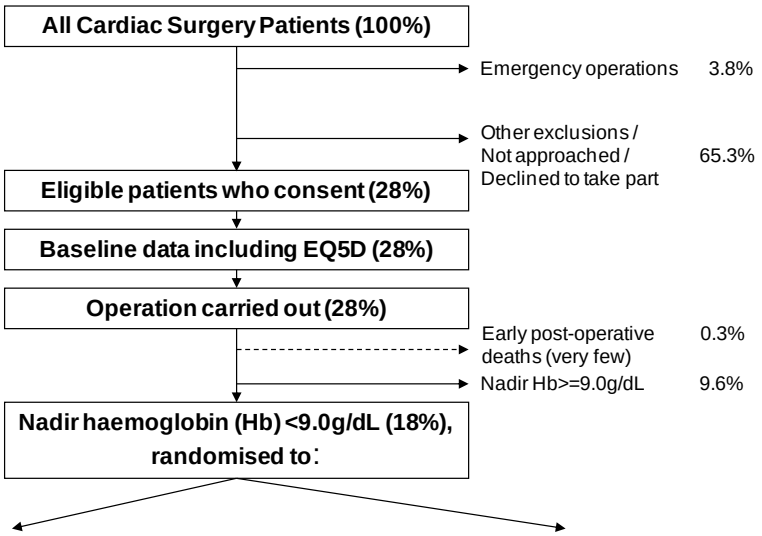
Because of these uncertainties, we propose a target sample size of 2000, i.e. 1000 participants in each arm of the trial. Because we expect approximately 2/3 of patients to breach the Hb threshold for eligibility (see below) we predict we need to register approximately 3000 patients into the study as a whole to allow 2000 patients to be randomised into the main study. No adjustment has been made for withdrawals or loss to follow-up, which is expected to be very low; none of the participants in TITRe 1 withdrew after randomisation.

The main outcome measure for the economic evaluation will be quality adjusted life years (QALYs) (see 3.20), which are measured on a continuous scale. The analysis of QALYs will include baseline QALYs as a covariate; the correlation between baseline and 3 month assessments of QALYs is assumed to be ≥0.3. With a total sample size of 2000, the trial will have >95% power to detect a standardised difference in continuous outcomes between groups of 0.2 with 1% significance (2-sided test). This magnitude of difference is conventionally considered to be “small”.[**31**]

Rates of transfusion in the trial will be critical to the success of the trial. Data for the distribution of nadir haematocrit from recent observational analysis are shown in **Figure 1**,**[1]** (see 3.3) both for the entire dataset (n=8,621, solid line) and for the most recent data (2003; n=1,106, dashed line). The most recent data show a slight shift in the cumulative frequency plot towards higher Hb, probably because of wider uptake of off-pump coronary artery bypass graft (CABG) (which causes less blood loss) over the period represented by the data. Data

describing nadir Hb were obtained specifically for the analysis and we have not linked the clinical and haematology databases for more recent years. However, the proportion of off-pump CABG has not continued to increase since 2003. Because Bristol has the highest proportion of off-pump CABG across the UK (and isolated CABG makes up ≈70% of the workload) and a low transfusion rate, we are confident that cumulative frequency plots for other centres would not lie further to the right (i.e. higher Hb). On this basis, we believe it is reasonable to assume that ≥65% of patients will breach the threshold of 9.0 g/dL, and ≈20% will breach the 7.5 g/dL threshold (see 3.3).

Figure 2: CONSORT diagram summarising TITRe 2 trial design.



	TRANSFUSION THRESHOLD		
	Nadir Hb <7.5g/dL	Nadir Hb ≥7.5 to <9.0 g/dL	Total
TRANSFUSED	96%	62%	74%
NOT TRANSFUSED	4%	38%	26%

	TRANSFUSION THRESHOLD		
	Nadir Hb <7.5g/dL	Nadir Hb ≥7.5 to <9.0 g/dL	Total
TRANSFUSED	96%	<5%	<35%
NOT TRANSFUSED	4%	>=95%	>=65%

Notes:

1. Percentages are based on data from the cardiac surgery registry in Bristol for the period Jan to Sep 2007, except for the proportion of patients and declining to take part and excluded for reasons other than age and emergency operation.
2. An unknown percentage of patients are excluded by the exclusion criteria because the registry does not contain sufficient detail to apply the definitions proposed for the trial. However, patients meeting one or more of these criteria are extremely rare and we expect all of the exclusion criteria to account for a maximum of 5% of cardiac surgery patients.
3. The largest estimated exclusion is for 'other exclusions' (see 5 above) and patients who are not approached or who decline to take part, 65.3%. The value assumed for the latter percentage is expected to be conservative since, across our trial portfolio (including the pilot trial), we have consistently recruited about 50% or more of the patients approached.
4. The percentages of patients transfused/not transfused in the two tables for randomised patients were used for the sample size justification and are based on our observational data.**[1]** Because the data are observational, we believe that the percentages, especially for the liberal threshold (left hand table), are conservative. In the trial, using the method described above for confirming with doctors that patients can be treated in accordance with the trial protocol, we would expect a higher percentage of patients to be transfused; if our hypothesis is correct, this would increase the observed difference in outcome.

3.15. Planned recruitment rate

Based on current expressions of interest, we aim to recruit from 12-14 cardiac surgery centres throughout the UK. Our worst case scenario is that only eight sites (see 0) take part. We would then need to randomise, on average, 250 participants from each site into the main study, with each site recruiting over 24 months. Based on data from TITRe 1, the pilot study for this trial, randomising 125 participants per year would require centres to randomise at most about 13% of all eligible patients into the main study. We anticipate that loss to follow-up will be very small, given the primary endpoint of 3 months after randomisation.

In practice we are likely to require more than eight centres to meet recruitment targets. Recruitment rates at different centres will be variable depending on factors such as the size of the centre, the number of surgeons participating and whether or not the centre is involved in any competing studies. Centres will be asked to estimate their predicted levels of recruitment on registering for the trial, to allow ongoing assessment of projected recruitment for the study and the need for additional centres.

We plan for two centres to be ready to recruit by July-August 2009, with other centres starting to recruit shortly after this. This will allow centre-specific activities such as obtaining local approvals and site initiation to be staggered. The flow of participants into the trial, the cumulative number of patients over time, and research activities during the trial are shown below in **Table 4**. Progress reports will be submitted to the funding body (the HTA) at 6-montly intervals, with the final report due to be submitted in December 2011.

3.16. Participating centre(s)

We aim to recruit 12-14 centres (including Bristol), each recruiting participants into the study over 24 months. The following centres have expressed interest in participating:

- Aberdeen (Aberdeen Royal Infirmary, NHS Grampian);
- Basildon (Essex Cardiothoracic Centre, Basildon and Thurrock Trust);
- Belfast (Royal Victoria Hospital Belfast Health and Social Care Trust);
- Blackpool (Lancashire Cardiac Centre, Blackpool Fylde & Wyre Hospitals NHS Foundation Trust);
- Brighton (Sussex Cardiac Centre, Brighton and Sussex University Hospitals NHS Trust);
- Bristol (Bristol Royal Infirmary, University Hospitals Bristol NHS Foundation Trust);
- Cardiff (University Hospital of Wales Cardiff, Cardiff and Vale NHS Trust);
- Coventry (University Hospitals Coventry & Warwickshire NHS Trust);
- Edinburgh (Royal Infirmary of Edinburgh, NHS Lothian);
- Glasgow (Golden Jubilee National Hospital, NHS Greater Glasgow and Clyde);
- Hull (Castle Hill Hospital, Hull and East Yorkshire Hospitals NHS Trust);
- Leeds (Leeds General Infirmary, Leeds Teaching Hospitals NHS Trust);
- Leicester (Glenfield Hospital, University Hospitals of Leicester NHS Trust);
- Liverpool (Liverpool Heart and Chest Hospital NHS Trust);
- Manchester (Wythenshawe Hospital, University Hospital of South Manchester NHS Foundation Trust);
- Middlesbrough (The James Cooke University Hospital, South Tees Hospitals NHS Foundation Trust);
- Newcastle (Freeman Hospital, The Newcastle-Upon-Tyne Hospitals NHS Foundation Trust);
- Plymouth (The Southwest Cardiothoracic Unit, Plymouth Hospitals NHS Trust);

- St George's Hospital (St George's Healthcare NHS Trust);
- Southampton (Southampton General Hospital, Southampton University Hospitals NHS Trust);
- UCL (The Heart Hospital, The UCLH NHS Foundation Trust);
- Wolverhampton (New Cross Hospital, Royal Wolverhampton Hospitals NHS Trust).

Table 4: Schedule of recruitment and other activities during the trial

				Trial month	Number of centres	Registered patients	Registered cumulative
Dec	2008	setup		1			
to							
Jun	2009	setup		7			
Jul	2009	recruit		8	1	3	3
Aug	2009	recruit	follow-up	9	1	10	13
Sep	2009	recruit	follow-up	10	2	8	21
Oct	2009	recruit	follow-up	11	2	36	57
Nov	2009	recruit	follow-up	12	4	40	97
Dec	2009	recruit	follow-up	13	5	38	135
Jan	2010	recruit	follow-up	14	5	27	162
Feb	2010	recruit	follow-up	15	5	53	215
Mar	2010	recruit	follow-up	16	6	62	277
Apr	2010	recruit	follow-up	17	7	66	343
May	2010	recruit	follow-up	18	7	67	410
Jun	2010	recruit	follow-up	19	7	70	480
Jul	2010	recruit	follow-up	20	8	50	530
Aug	2010	recruit	follow-up	21	9	64	594
Sep	2010	recruit	follow-up	22	9	59	653
Oct	2010	recruit	follow-up	23	11	73	726
Nov	2010	recruit	follow-up	24	11	101	827
Dec	2010	recruit	follow-up	25	11	45	872
Jan	2011	recruit	follow-up	26	11	82	954
Feb	2011	recruit	follow-up	27	13	85	1039
Mar	2011	recruit	follow-up	28	13	74	1113
Apr	2011	recruit	follow-up	29	13	74	1187
May	2011	recruit	follow-up	30	14	88	1275
Jun	2011	recruit	follow-up	31	14	87	1362
Jul	2011	recruit	follow-up	32	14	104	1466
Aug	2011	recruit	follow-up	33	14	131	1597
Sep	2011	recruit	follow-up	34	14	133	1730
Oct	2011	recruit	follow-up	35	14	118	1848
Nov	2011	recruit	follow-up	36	14	104	1952
Dec	2011	recruit	follow-up	37	14	128	2080
Jan	2012	recruit	follow-up	38	15	128	2208
Feb	2012	recruit	follow-up	39	16	128	2336
to							
Dec	2012	recruit	follow-up	49	16	128	3616
Jan	2013	Recruit	follow-up	50	16	128	3744
Feb	2013	analyse	follow-up	51	16		3744
Mar	2013	analyse	follow-up	52	16		3744
Apr	2013	analyse	follow-up	53	16		3744
May	2013	analyse	follow-up	54	16		3744
Jun	2013	analyse	write-up	55			
to							
Nov	2013	analyse	write-up	60			

1 Numbers of centres and registered patients are actual numbers up to November 2011, then projected numbers up to January 2013 based on the current target recruitment rate.
2 Data validation and cleaning will be carried out throughout the trial, as data are entered into the database. Queries about suspect or missing data will be fed back to centres at least weekly, within one week of data submission and entry into the database.
3 Analysis programmes will be developed during the last year of the trial, but without access to the data designating random allocation of participants to alternative threshold groups.
4 The final report will be drafted during the last 6-9 months of the trial, including dummy tables and figures, so that finalising the report can be carried out promptly once the final analyses are available.

Although the number of non-emergency operations carried out each year varies to some extent across these centres, such variation will not restrict the proposed recruitment rate. For example, the number per year in Bristol (from data collected for TITRe 1) is about 1,300 per year, of whom about 1,100 are eligible, and Bristol is not the largest centre.

We anticipate that the decision to participate can be made by lead clinicians in a centre and their managers, who will need to agree that randomisation is acceptable and that the 'fee per patient randomised' (see 4.3) is sufficient to cover the research coordination / staff time required to recruit patients and collect data in accordance with the protocol and good clinical practice guidelines.

We have obtained information about current transfusion thresholds and the proportion of patients currently transfused from five centres that have expressed interest in the trial (see **Table 5**).

Table 5: Transfusion thresholds and proportion of patients currently transfused for five centres expressing an interest in the TITRe2 trial

Centre	Transfusion threshold	Percentage transfusion
Basildon	8.0 g/dL	50% (3 month audit in 2007)
Bristol	7.5 to 8.0 g/dL	33% (Jan to Sep 2007, incl.)
Cardiff	8.0 g/dL	44% (cardiac registry, 2005-6)
Edinburgh	8.0 g/dL	50% (cardiac registry, 2007)
Liverpool	No fixed protocol (7.0 to 9.0 g/dL across surgeons)	23% to 75% across surgeons

Despite the assertions of centres that they transfuse in accordance with local protocols, we suspect that much transfusion practice remains inconsistent; this was demonstrated by the audit in Basildon, which showed that the actual nadir Hb leading to transfusion was 8.5 g/dL. It is also important to note that overall transfusion rates reported by centres (given the stated transfusion thresholds) suggest either more liberal [actual] transfusion practice or cumulative frequency nadir Hb plots which are shifted further to the left than observed in Bristol (**Figure 1**). In Bristol, only 36.7% of patients in 2003 had a Hb below 8.0 g/dL and this agrees quite well with the overall percentage transfused in Jan to Sep 2007; however, there is no guarantee that the patients with a nadir Hb <8.0 g/dL are the ones who are being transfused.

3.17. Investigators' responsibilities

Investigators will be required to ensure that all necessary research governance approvals have been obtained and that any contractual agreements required have been signed off by all parties prior to the start of the study. Investigators will be required to ensure compliance to the protocol and study manual and with completion of the CRFs. Investigators will be required to allow access to study documentation or source data on request for monitoring visits and audits performed by the Sponsor or study co-ordinating centre (CTEU) or any regulatory authorities.

The Principal Investigator at each participating centre has overall responsibility for the study and all patients entered into the study at that site, but may delegate responsibility to other members of the study team as appropriate. The Principal Investigator must ensure that all staff involved are adequately trained and their duties have been logged on the Site Signature and Delegation Log, which will be provided by the co-ordinating centre.

3.18. Training and monitoring

Pre-study training visit

Before the study commences each centre will receive a training visit by the study co-ordinating centre. These visits will ensure that personnel at each site (including principal investigators, co-investigators and the study site co-ordinator) fully understand the protocol, CRFs and the practical procedures for the study.

Monitoring of participating centres

Participating centres will be monitored by staff from the co-ordinating centre as the trial progresses to confirm compliance with the protocol and the protection of patient's rights. Participating centres will be monitored by checking incoming data for compliance with the protocol, consistency, missing data and timing. All queries will be fed back to centres. Study staff at the co−ordinating centre will be in regular contact with site personnel (by phone / fax / email / letter) to check on progress and deal with any queries they may have.

Routine on−site monitoring visits to each centre by the coordinating centre are not planned. It has been suggested that on-site monitoring is an inefficient way to identify errors most likely to compromise patient safety or bias study results.[**32**] Central monitoring of submitted data is more likely to lead to tangible benefits,[**32**] is less costly and represents a more efficient use of trial personnel.

However on-site monitoring visits will be carried out if a site requests a visit, data quality suggests it is necessary or other concerns are raised. The need for on−site monitoring at individual sites will be reviewed on an ongoing basis. If on−site monitoring takes place, CRFs for a sample of participants will be audited against medical records. Under existing NHS Research Governance arrangements, sites may also be monitored by their own, local R&D Departments.

3.19. Plan of analysis

All primary analyses will be based on the intention-to-treat. Secondary, observational analyses comparing groups of patients who are transfused or not will also be carried out; the findings of these analyses will be compared for consistency with previous findings.[**1**]

Analyses of primary outcome

This outcome will by analysed by logistic regression. Subgroup analyses will be carried out for the following groups: (i) operation type (isolated CABG vs. other operation types); (ii) age at operation (<75 vs. ≥75 years); (iii) preoperative diagnosis of diabetes or not; (iv) preoperative diagnosis of lung disease (chronic obstructive airways disease or asthma) or not; (v) preoperative renal impairment (preoperative creatinine >177µmol/L) or not (vi) sex, (vii) ventricular function (good versus moderate or poor). These interactions will be carried out to test widely-held beliefs such 'at-risk' groups should be transfused at higher Hb thresholds because of their vulnerability. The prior hypothesis for all subgroup analyses is that there will be no interaction. These analyses will carried out by Cox regression if dates of events are successfully collected, as planned.

Analyses of secondary outcomes

The proportions of patients experiencing an infectious or an ischaemic event (secondary outcomes) will also be analysed separately, by logistic regression. No subgroup analyses will

be carried out. The frequencies of individual components of the primary outcome will be tabulated.

Analyses of intensive care or high dependency unit stay, overall length of hospital stay and all cause mortality

Analyses of these secondary outcomes will be carried out using Cox regression, censoring any participants who are lost to follow-up at the last known follow-up. We anticipate that very few patients will be lost, given the short duration of follow-up. No subgroup analyses will be carried out.

Analyses of the number of units of red cell transfused and EuroQol EQ5D

Units of red cells transfused (transformed), and utilities assigned on the basis of responses to the EuroQol EQ5D questionnaire, will be analysed by linear regression.

Frequency of analyses

We propose that an interim analysis should be carried out after 50% of patients have been recruited and followed for 3 months (~month 18-20). The details of this analysis, and actions contingent on the results, will be decided by the DMEC before starting recruitment and will be forwarded to the REC.

Other prespecified analyses

Three additional observational analyses will be carried out:

1. Several previous observational studies have found a dose-response relationship between the number of RBC units transfused and the risk of mortality and morbidity.[**33**] TITRe2 gives the opportunity to test the relationship in a RCT, specifically to estimate the dose-response relationship stratified by trial arm.
2. There is evidence that some of the mortality risk associated with RBC transfusion can be attributed to the 'age' of RBC, i.e. time from donation to transfusion.[**34**] Therefore, we will investigate whether RBC age is associated with the risk of both primary and secondary outcomes.
3. The level at which a low Hb increases the risk of postoperative mortality and morbidity varies widely, not just between individuals but also for any one individual during the perioperative period.[**33**] Quantifying the percentage decline in Hb from the preoperative level, rather than applying a generic Hb threshold, may provide a very simple way of setting an individualised threshold for a patient. Therefore, we will investigate the relationship between the percentage decline in Hb and the risk of primary and secondary outcomes, taking into account the number of RBC units transfused.

3.20. Economic issues

Established guidelines will be used for the conduct of the economic evaluation.[**35,36**] The main outcome measure for the economic evaluation will be QALYs, estimated using the EuroQol EQ5D.[**30**] This questionnaire instrument will be administered face-to-face at baseline and posted to randomised participants to complete at 6 weeks and all registered participants at 3 months. Respondents will be assigned valuations derived from published UK population tariffs[**37**] and the mean number of QALYs per trial arm and incremental QALYs will be calculated.

Data will be collected from the trial centres on health care resource use for transfusion, any complications, and subsequent treatments for complications. Resource use will be measured in naturally occurring units; for example, staff time will be measured in terms of length of times for treatments and unit costs will be derived from nationally published sources. Costs for further contact with health care professionals such as GP visits and patient travel costs will

be estimated using a custom designed questionnaire (adapted from similar questionnaire used for other trials of hospital interventions).

The analysis will calculate the average cost and outcome on a per patient basis and from this the incremental cost-effectiveness ratios for the different trial arms will be derived, producing an incremental cost per QALY (and other economic outcome measures, such as infectious or ischaemic event). Probabilistic sensitivity analysis will be used to demonstrate the impact of the variation around the key parameters in the analysis on the baseline cost-effectiveness results. Results will be expressed in terms of a cost-effectiveness acceptability curve, which indicates the likelihood that the results fall below a given cost-effectiveness ceiling.

4. Study Organisation

4.1. Sponsorship

University Hospitals Bristol NHS Foundation Trust (UHBristol) will be acting as the Sponsor for the study.

4.2. Co-ordinating centre

The study co-ordinating centre will be the Clinical Trials and Evaluation Unit (CTEU), Bristol Heart Institute, University of Bristol.

4.3. Funding arrangements

The trial is funded by a project grant from the National Institute for Health Research Health Technology Assessment Programme (NIHR HTA). The research grant, which is held by the University of Bristol, includes a per-patient payment for Participating NHS Trusts / Boards of £160 per patient randomised into the main study. Payments will be administered by the University of Bristol and payment schedules will be detailed in a study agreement with participating NHS Trusts / Boards. In addition, as this is an NIHR CRN portfolio study (see 4.4) participating centres are eligible for service support costs from their local Comprehensive Research Network (English sites) or R&D department (other UK sites).

4.4. NIHR Clinical Research Network (CRN) portfolio adoption

The study has been adopted onto the NIHR CRN (formally UKCRN) portfolio of clinical research (UKCRN ID 5338).

5. Project management

5.1. Day-to-day management

The trial will be managed by a Trial Management Group (TMG), which will 'meet' approximately monthly by teleconference. The TMG will be chaired by the Chief Investigator and will consist of the applicants and other members of staff from the trial co-ordinating centre (including the trial manager). Two or three representatives from participating centres will be invited to attend TMG meetings.

The trial will be overseen by a Trial Steering Committee and a Data Monitoring and Ethics Committee (see below). Both Committees will be convened before the start of recruitment.

5.2. Trial Steering committee and Data Monitoring and Ethics Committee

Trial Steering Committee (TSC)

Membership (n=8/9) of the TSC will include:

- An independent chair and cardiac surgery specialist (Professor John Pepper, Royal Brompton Hospital);

- A lay/consumer representative (from the Research Advisory Group for the Bristol Heart Institute) (Karin Smyth)
- An independent intensive care specialist (Dr Duncan Young, Oxford Radcliffe Hospitals NHS Trust);
- An independent haematology specialist (Dr Edwin Massey, NHS Blood Transplant, NBS Bristol);
- An independent statistician / trialist (Dr Gordon Taylor, University of Bath)
- Representatives of TITRe 2 (Professor Reeves, Mr Murphy);
- A representative of the trial sponsor, the United Bristol Healthcare Trust will be invited to TSC meetings.
- A representative of the HTA programme will be invited to TSC meetings.

Data Monitoring and Ethics Committee (DMEC)

Membership (3 members) of the DMEC will include:
- An independent chair and trialist / statistician (Professor Gordon Murray, University of Edinburgh)
- An independent intensive care specialist (Professor Tim Walsh, Royal Infirmary of Edinburgh;
- An independent cardiac surgery specialist (Mr Domenico Pagano, University Hospital Birmingham)

6. Safety reporting

6.1. Definitions

- An ***adverse event (AE)*** is any untoward medical occurrence in a subject receiving treatment according to the protocol, including occurrences which are not necessarily caused by or related to administration of the research procedures.
- A ***serious adverse event (SAE)*** is defined as an AE that:

  (a) results in death

  (b) is life-threatening

  (c) requires hospitalisation or prolongation of existing hospitalisation

  (d) results in persistent or significant disability or incapacity

Notes:

1) Life threatening in the definition of an SAE refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

2) Medical judgement should be exercised in deciding whether an AE is serious in situations not listed in (a) to (d) above.

Participants in the study are undergoing major heart surgery. Therefore many AEs, including death, are expected (see section 6.3 for details).

- An ***unexpected SAE*** is defined as any AE meeting the definition of an SAE above, and that is not listed in the protocol as an 'expected occurrence not subject to expedited reporting' (see section 6.3 below).
- An ***unexpected related SAE*** is defined as an unexpected SAE that is judged to be possibly, probably or definitely related to allocation to one or other arm of the trial, i.e. having or withholding transfusion in accordance with the protocol.

6.2. SAE reporting

SAEs will be recorded and reported in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines and the Sponsor's Research Related Adverse Event Reporting Policy.

SAE reporting by participating sites

- All fatal or unexpected SAEs must be notified by participating sites to the TITRe2 co-ordinating centre using the SAE form supplied within 24 hours of the site identifying the event.
- Multiple SAEs may occur in a single participant. Details of all SAEs for a participant, including the order in which multiple SAEs occurred, will be collected on the relevant case report forms (in the case of multiple unexpected or fatal SAEs, information for each SAE will be collected on individual SAE forms).

SAE reporting by the TITRe 2 co-ordinating centre and study sponsor

- The TITRe2 co-ordinating centre will notify all deaths, and all unexpected non-fatal SAEs to the Trial Sponsor (UHBristol R&D Office).
- Data on *unexpected related* SAEs will be reported by the sponsor to the research ethics committee within 15 days as required by the regulatory authorities
- Data on all AEs and SAEs will be collated by the TITRe2 trial coordinating centre, and reported regularly to the Data Monitoring and Ethics Committee (DMEC), distinguishing carefully SAEs that occur in the same participants.

6.3. Events not subject to expedited reporting (expected occurrences)

The following events are expected occurrences in cardiac surgery patients, and are not subject to expedited reporting to the sponsor (except for fatalities). In circumstances where a fatality is possibly, probably or definitely related to allocation to one or other arm of the trial, the fatality will be reported by the sponsor to the regulatory authorities. SAEs within 3 months for an expected occurrence listed below are also not subject to expedited reporting (unless the SAE results in a fatality).

- Any element of the infectious / ischaemic events (listed in Table 1, Section 3.11) as part of the composite primary outcome, including:
    - Sepsis
    - Wound infection
    - Permanent stroke
    - Myocardial infarction
    - Acute kidney injury
    - Gut infarction
- Transient ischaemic attack
- Other gastro-intestinal complications, including:
    - Pancreatitis
    - Obstruction or perforation
- Post-operative haemorrhage
- Cardiac tamponade
- Pulmonary complications, including:
    - Acute respiratory distress syndrome
    - Re-intubation and ventilation
    - Tracheostomy
    - Initiation of mask continuous positive airway pressure ventilation after weaning from ventilation
    - Pneumothorax requiring chest drainage
    - Pleural effusion requiring drainage

- Arrhythmias, including:
    - Supraventricular tachycardia or atrial fibrillation requiring treatment
    - Ventricular fibrillation or tachycardia requiring intervention
    - Pacing
- Re-operation for any reason, including:
    - Bleeding
    - Cardiac arrest
    - Mediastinitis
- Thromboembolic complications, including:
    - Deep vein thrombosis
    - Pulmonary embolus
- Low cardiac output, requiring management with a Swan-Ganz catheter, an intra-aortic balloon pump, or left ventricular assist device
- Wound dehiscence requiring rewiring or treatment for reason other than infection
- Death

Any SAE (section 6.1) not listed above will be treated as an 'unexpected' SAE and will be reported to the TITRe 2 co-ordinating centre using the study SAE report forms (within the CRF). All deaths (irrespective of any preceding SAEs) will also be reported to the TITRe 2 co-ordinating centre. All unexpected SAEs and deaths will be reported to the REC.

6.4. Period for recording SAEs

Data on SAEs will be collected for randomised participants only, from the time of randomisation into the main study and for the duration of the participant's post-operative hospital stay and the 3 month follow-up period.

All SAEs occurring during the participant's post-operative hospital stay should be notified by the participating site to the TITRe 2 co-ordinating centre, as described in sections 6.2 and 6.3 above. If a participating site becomes aware of an SAE occurring in a trial participant after discharge from hospital and within the 3 month follow-up period, they must notify the TITRe 2 co-ordinating centre as described in sections 6.2 and 6.3 above.

Data on surgical complications and other adverse events occurring after discharge will be collected via the postal follow-up questionnaires administered by the TITRe 2 co-ordinating centre at 3-months post-randomisation. Further details of any event identified at this time that are (a) suspected to meet the definition of SAE (whether expected or unexpected), or (b) suspected to contribute to the primary outcome, will be sought, e.g. from the admitting hospital or the participant's general practitioner. Any such event will be reported to the sponsor and regulatory authorities if required (see sections 6.2 and 6.3 above). Participating sites may be asked to supply the co-ordinating centre with information on these events as required.

7. Ethical approval, research governance and indemnity

7.1. Ethical review

The study protocol has been reviewed and approved by the 'Oxfordshire Research Ethics Committee (REC) C' (REC ref: 08/H0606/125).

With respect to equipoise about the main research questions there is:
- evidence from surveys of diverse practice between clinicians and centres;
- evidence from RCTs of net benefit to patients having other (non-cardiac) major surgery from using a restrictive transfusion threshold;
- evidence from observational studies of serious harms associated with transfusion.

7.2. Consumer involvement

TITRe 2 has been discussed by the Bristol Heart Institute's Research Advisory Group, which includes key stakeholders with an interest in the research carried out by the Bristol Heart Institute (patients, charities representing patients' interests, general practitioners, NHS commissioners, and a regional cardiac network). Patient representatives from the Research Advisory Group have been consulted in the design of patient documents for the study. A lay consumer representative from the Research Advisory Group is a member of the TSC.

7.3. Research governance

This study will be conducted in accordance with:

- Relevant aspects of the Medicine for Human Use (Clinical Trial) Regulations 2004
- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines
- Research Governance Framework for Health and Social Care

Local Research and Development (R&D) approval in the UK requires that the trial be conducted in compliance with the Research Governance Framework.

7.4. Clinical Trial Authorisation

The MHRA has confirmed that this trial does not require a Clinical Trial Authorisation.

7.5. Risks and anticipated benefits

Potential benefits to participants include the possibility of a reduction in the risk of life-threatening transfusion-associated post-operative complications in the group randomised to a restrictive transfusion threshold. Should our hypothesis be supported by the findings of the trial, all future patients will benefit from the reduced risk of complications. The main benefit to society is the provision of high quality evidence to address this important area of clinical uncertainty. In addition, if our hypothesis is supported by the findings, the NHS should benefit from gains in efficiency arising from reductions in length of stay in intensive care and on cardiac wards (both expensive and scarce resources), as well as saving the costs of blood that is not used for transfusion and treatments for complications which are avoided.

Potential harms to participants include the possibility of randomisation to an inferior treatment (a possible harm of participating in any trial) and, for those allocated to the restrictive threshold, possible side effects of not having transfusion when transfusion might have benefits. The 'reasonableness' of asking participants to accept the possibility of randomisation to an inferior treatment (i.e. the prevailing uncertainty about the research questions of interest and the benefits and risk of carrying out the trial to participants, future patients and society) has already been judged by our application to the NHS REC for ethical approval for the trial.

7.6. Informing potential study participants of possible benefits and known risks

Information about possible benefits and risks of participation will be described in a Patient Information Sheet (PIS) sent or given to patients before they are admitted for surgery. This PIS has been approved by the REC giving ethical approval for the trial.

7.7. Obtaining informed consent from participants

All participants will be required to give written informed consent. The process for obtaining informed consent, including the information in the PIS, was described in our application to the REC for ethical approval. Whenever possible, patients will be identified during their first outpatient appointment by the surgeon or a member of the research team for potential

inclusion in the trial. Following the outpatient appointment, a copy of the Patient Information Leaflet will be posted to the patient. Whenever possible, the patient will subsequently be approached at the pre-assessment clinic where a member of the research team will discuss the trial, answer any questions, and assess whether or not the patient wishes to participate in the study. The patient may also be contacted by a follow up telephone call after the Patient Information Leaflet has been posted to them to ascertain their interest in the clinical trial. If the patient does express an interest then, as part of the final assessment or admission process prior to surgery, he/she will be approached for consenting and recruitment. If for any reason the patient is not attending a local pre-assessment clinic, the patient will be approached at the time they are admitted for surgery, when a member of the research team will discuss the trial, answer any questions, and assess whether or not the patient wishes to participate in the study as well as consent the patient.

Patients requiring urgent surgery are often cared for in outlying hospitals until their operations can be carried out. When these patients are referred for surgery (without having an outpatient appointment or attending a pre-assessment clinic, the research team will fax the PIL to the referring hospital. Whenever the PIL is faxed, a member of the research team will endeavour to ring the ward and to speak to a member of staff or the patient to confirm that the PIL has been received and to answer any questions the staff or patient may have. The patient will be told that he/she will be approached to discuss the study once they are transferred for surgery.

In a few cases, the time interval between receiving the PIL and formal consent being sought may be as little as 12 hours, for example for patients admitted for urgent surgery without prior notification to the waiting list co−ordinator. Despite the short notice, it is important to include these patients for the applicability of the trial findings.

7.8. Research procedures

Participants will be required to do, or undergo, the following tasks or investigations specifically for the research:

- Read a PIS about the study;
- Give written consent to participate if willing to do so;
- Complete the EQ5D questionnaire before their operation;
- Have additional blood tests as required to comply with local transfusion procedures and the study protocol, e.g. blood group test and antibody screen; wherever possible these will be taken using catheter lines already inserted;
- Allow surgical wounds to be assessed as required for completing the ASEPSIS instrument during the hospital stay;
- Complete and return by post a questionnaire providing information about any surgical complications and other adverse events experienced after discharge;
- Complete and return by post the ASEPSIS post-discharge surveillance questionnaire;
- Complete and return by post the follow-up EQ5D questionnaire which will be sent to participants on two occasions.

Other data about participants' usual care during their admissions will be collected and used for the research.

7.9. Monitoring and audit by the sponsor

The study will be monitored and audited in accordance with the Sponsor's policy, which is consistent with the Research Governance Framework and the Medicines for Human Use (Clinical Trials) Regulations 2004. All study related documents will be made available on request for monitoring and audit by the sponsor or the REC.

The trial coordinating centre will carry out regular monitoring and audit of compliance of centres with GCP and data collection procedures as per 3.18 above)

7.10. Data protection

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

7.11. Data storage and sharing

Data storage

We will retain all study documentation in a secure location during the conduct of 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, the fully pseudo-anonymised dataset, a separate secure electronic 'key' with a unique patient identifier, and relevant 'meta'-data about the trial be retained in electronic form indefinitely because of the potential for the raw data to be used subsequently for secondary research.

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.

7.12. Indemnity

This is an NHS-sponsored research study. For NHS sponsored research HSG(96)48 reference no. 2 refers. If there is negligent harm during the clinical trial when 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.

8. Dissemination of findings

The findings will be disseminated by usual academic channels, i.e. presentation at international meetings, as well as by peer-reviewed publications and through patient organisations and newsletters to patients, where available. We will also consult our Research Advisory Group about other ways of disseminating the findings to patients and other stakeholders.

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10. List of Abbreviations

A&E - Accident & Emergency
AE - adverse event
AKI - Acute Kidney Injury
AKIN - Acute Kidney Injury Network
ASEPSIS - (Scoring system) additional treatment, serious discharge, erythema, purulent exudate, separation of deep tissues, isolation of bacteria, stay duration as inpatient
CABG - coronary artery bypass graft
CICU - Cardiac Intensive Care Unit
CONSORT - Consolidated Standards of Reporting Trials
CRF - Case Report Form
CRN - Clinical Research Network
CT - Computerised Tomography
CTEU - Clinical Trials and Evaluation Unit
DMEC - Data Monitoring and Ethics Committee
GCP - Good Clinical Practice
GP - General Practitioner
Hb - Haemoglobin
HCT - Haematocrit
HDU - High Dependency Unit
HTA - Health Technology Assessment
ICH GCP - International Conference for Harmonisation of Good Clinical Practice
ICU - Intensive Care Unit
ID - Identification
MHRA - Medicines and Healthcare Products Regulatory Agency
MI - Myocardial Infarction
MRC - Medical Research Council
MRI - Magnetic Resonance Imaging
MRIS - Medical Research Information Service
NIHR HTA - National Institute for Health Research Health Technology Assessment Programme
PIS - Patient Information Sheet
QALYs - quality adjusted life years
QoL - Quality of Life
R&D - Research & Development
RBC - Red Blood Cell
RCT - Randomised Controlled Trial
REC - Research Ethics Committee
RRT - Renal Replacement Therapy
SAE - serious adverse event
SIRS - Systemic Inflammatory Response Syndrome
TMG - Trial Management Group
TRALI – Transfusion related acute lung injury
TRICC - Canadian Transfusion Requirements in Critical Care
TSC - Trial Steering Committee
UHBristol - University Hospitals Bristol NHS Foundation Trust
Vcjd - Variant Creutzfeldt-Jakob disease
WBC - White Blood Cell

11. Summary of protocol amendments

The following amendments and/or administrative changes have been made to the protocol since the date of original REC approval (original version approved: protocol v2.0, 10th September 2008) and subsequent approved version (previous version approved: protocol v3.0, 21st April 2009).

Previous version	Previous date	New version	New date	Summary of key changes	Date of ethical approval (or NA if non-substantial)
2.0	10/09/2008	3.0	21/04/2009	• Addition of Hct values (corresponding to Hb values already listed) to be used in trial transfusion protocols • Amendments to eligibility criteria • Amendments to definitions of infectious / ischaemic events for primary outcome • Update to 'Safety Reporting' section to clarify SAE reporting procedures • Addition to list of SAEs classified as 'expected occurrences'	19/05/2009
3.0	21/04/2009	4.0	24/06/2009	• Addition to list of SAEs classified as 'expected occurrences'	14/07/2009
4.0	24/06/2009	5.0	07/09/2009	• Amendment to transfuse with RBC within 24 h of Hb/Hct dropping below allocated threshold • Amendment to follow-up for non-randomised patients • Amendments to list of SAEs classified as 'expected occurrences'	09/10/2009
5.0	07/09/2009	6.0	08/03/2010	• Clarification that the most recent Hb/Hct reading should be used as a trigger for transfusion within 24h • Amendment to clarify that patients with iron-deficient anaemia can be included in the study • Amendment to include Troponin T in addition to I as some centres participating in the study use T rather than I. As part of this change we have removed the threshold for	15/04/2010

				MI in the protocol, the highest troponin reading for all patients with suspected MI will be collected and a threshold for MI will be decided when reviewing the complete dataset. • Addition of 3 new sites, we now anticipate that 12-14 sites will be needed to meet target recruitment. • Removal of note 3 in section 6.1 (Safety Reporting – definitions)	
6.0	08/03/2010	7.0	24/06/2010	• Removal of the upper age limit for inclusion of patients. • Clarification the sealedenvelope requires a pin in addition to the password. • Clarification about the procedures used to remind patients to complete follow-up questionnaires. • Clarification of which SAEs should be reported to the Sponsor and how information about readmissions will be obtained.	04/08/2010
7.0	24/06/2010	8.0	03/03/2011	• Removal of "events causing A&E admission" from primary outcome • Deletion of specific list of surgical wound sites from ASEPSIS • Clarification that the definition for sepsis is for the index admission • Clarification that troponin will be reviewed before the study database is locked • Added TACO to list of secondary outcomes • Updated that further details will be sought for events causing readmission, it is not always possible to access medical notes from other hospitals. • Clarified that PIs will be expected to ensure that all necessary research governance approvals are in place. • Clarified AE/SAE reporting procedures	

				• Added cardiac tamponade as “expected” event. • Added that additional blood tests may be required to comply with local transfusion protocols.	
8.0	03/03/2010	9.0	12/02/2012	• Adapt the 3-month telephone questionnaire to a postal questionnaire. Telephone questionnaires will still be used in cases where the patient prefers it, or there is no response to the postal questionnaire • Approaching patients in the pre-assessment clinics to discuss the trial • Added 2 new references • Sending patients in a referring hospital awaiting surgery a PIL by fax • Change of Chair of the Trial Steering Committee • Inclusion of Coventry as a site • Deleted TACO as a secondary outcome and added significant pulmonary morbidity • Changes to the classification of serum creatinine for acute kidney injury • Additional information on Evidence about transfusion thresholds •	