Pharmacokinetics and Pharmacodynamics of Platelet $P2Y_{12}$ Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction: A Pilot Study

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Protocol authorised by:

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Study Management Group

Chief Investigator:	Dr J Cotton
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Clinical Queries

Clinical queries should be directed to Dr James Cotton who will direct the query to the appropriate person.

Sponsor

The Royal Wolverhampton NHS Trust is the research sponsor for this study. For further information regarding the sponsorship conditions, please contact R&D Department, The Royal Wolverhampton NHS Trust– Tel: 01902 695065 or email: lorraine.jacques@nhs.net

Funder

This study has been part funded by the Royal Wolverhampton Hospitals NHS Trust Pharmacy Department.

This protocol describes the above study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study.
Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance

with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

CONTENTS

GLOSSARY OF ABBREVIATIONS AND TECHNICAL TERMS

ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AE	Adverse event
CABG	Coronary artery bypass graft
ECG	Electrocardiogram
HTPR	High on-treatment platelet reayctivity
NSTEMI	Non-ST segment elevation myocardial infarction
PPCI	Primary percutaneous coronary intervention
SAE	Serious adverse event
STEMI	ST-segment elevation myocardial infarction
TIMI	Thrombolysis in myocardial infarction
UA	Unstable angina
VASP	Vasodilator stimulated phosphoprotein phosphorylation

KEYWORDS

Pharmacodynamic
Pharmacokinetic
Antiplatelet
Myocardial infarction
Clopidogrel
Prasugrel
Ticagrelor
STEMI
NSTEMI

1. LAY SUMMARY

Major heart attacks are caused by a number of factors, the two major of which are furring up of a coronary artery with atheroma and then sudden clot formation on this area leading to a blockage and interruption of blood flow. The clots that lead to heart attacks are largely made of clotting blood cells (platelets) that in health repair blood vessels and inhibit spontaneous bleeding. One of the main treatment strategies for heart attacks is to make these cells less "sticky". Aspirin is a main stay of treatment at our institution and in addition one of three other antiplatelet agents acting on the same platelet activation pathway ($P2Y_{12}$ receptor) are used (clopidogrel, prasugrel or ticagrelor). When a patient is admitted with a major heart attack, they are treated with emergency primary percutaneous coronary intervention (PPCI) a technique where a wire and balloon are used to reopen the coronary artery and then usually a stent (a slotted metal tube) is placed to keep the artery open. Aspirin and one of the $P2Y_{12}$ inhibitor agents are given to prevent further clots and all have been shown to reduce negative events following heart attacks and angioplasty with stent insertion.

All three $P2Y_{12}$ inhibitor agents are taken in tablet form immediately before the emergency PPCI procedure. It appears that these agents in healthy stable patients take at least 30 min to 2 hours to have an adequate effect. In PPCI patients, often the procedures are performed well within this timescale. Furthermore patients who are having a heart attack may not have normal drug absorption with blood being diverted away from the stomach and gut activity being suppressed by other drugs such as morphine.

In this study patients with major heart attacks will be given one of the three anti-platelet agents (being treated as per normal clinical pathways). Blood samples will be taken to assess the effect of the anti-platelet agents when the balloon opens the artery and after 20 minutes, 60 min and 4 hours after taking the drugs.

A variety of tests will be used to check the activity of the drugs including measuring the concentration of the active component of each of the three anti-platelet agents in the blood.

In total the patients in the study will require up to 4 extra blood tests, each using 15 ml of blood (approximately 3 teaspoons of blood). This will not cause any ill effects but may involve up to 3 extra needle pricks.

One of the major negative occurrences after PPCI is clotting within the stent- this occurs in 0-2% of cases and in other patients is associated with a poor response to these anti-platelet drugs. It is possible that this study will identify which, if any, of the agents used are working during the procedure, given the very short timescales involved. In the future it might be that an intravenous agent will be more valuable in the setting of PPCI.

2. INTRODUCTION

2.1 BACKGROUND

The success of modern treatment for ST-segment elevation myocardial infarction (STEMI) is dependent, in part, on the adequate suppression of platelet activity. Primary percutaneous coronary intervention (PPCI) for STEMI management has led to marked reductions in morbidity and mortality (1). Aspirin is the mainstay anti-platelet agent (2) which acts by irreversible acetylation and inhibition of the COX-1 enzyme, thereby reducing the production of thromboxane A2, a potent platelet activator. The advent of the platelet $P2Y_{12}$ inhibitors led to further improvement in outcomes in patients with acute coronary syndromes (ACS) undergoing PCI with reduced ischaemic complications (3) albeit at an increase in rate of bleeding complications. Both of these agents have a synergistic effect on platelet function with the addition of clopidogrel to aspirin leading to approximately 20% reduction in negative cardiovascular events (4). Activation of platelets leads to a conformational change in the surface glycoprotein IIb/IIIa receptor that allows cross bridging with fibrinogen and rapid aggregation (platelet thrombus formation).
During PPCI thrombus formation and dispersion can have a major impact on outcome. Intravenous platelet IIb/IIIa receptor antagonists such as abciximab were initially shown to have a positive impact on primary angioplasty outcomes leading to a class IIa indication within the European Society of Cardiology (ESC) guidelines for use in the treatment of STEMI with angioplasty (5).
As oral antiplatelet inhibition has improved (specifically with the addition of clopidogrel, prasugrel or ticagrelor) there has been a concomitant reduction in the use of intravenous IIb/IIIa agents during PCI for patients with ACS. This has been partly informed by later studies suggesting that the additive value of these agents to dual anti-platelet therapy is questionable (6). In addition, the extra bleeding complications are seen by some to be excessive (7).

Clopidogrel, the most commonly used $P2Y_{12}$ inhibitor has been shown to reduce ischaemic complications in patients with ACS both with and without PCI (8), but there are significant issues regarding both the uniformity of action across patients (9) and also the speed of onset of action of this agent (10). There are a variety of causes for the variable response to clopidogrel amongst subjects, leading to high on-treatment platelet reactivity (HTPR), but key is the two stage hepatic conversion to its active metabolite with variances in the CYP 450 isoenzymes (particularly 2C19) playing a major role.
As many as 40 % of treated patents may have a suboptimal response to clopidogrel and these patients are more likely to suffer recurrent ischaemic events and in particular stent thrombosis (11). Notably one of the first papers demonstrating this problem involved PPCI patients (12). Further large scale trials have identified the risk of poor clopidogrel activity with negative outcomes in PPCI patients (13).

Prasugrel, a newer thienopyridine has a greater uniformity of action and a more rapid onset of action than clopidogrel. This appears to lead to improved outcomes in patients

treated with PCI for ACS and in particular a reduction in stent thrombosis (TRITON-TIMI 38).(14). NICE guidelines (TA 182) now recommend the use of prasugrel in treatment of patients either over the age of 75 or over 60kg undergoing PPCI.
The TIMI-TRITON 38 trial was however biased toward prasugrel in that the majority of drug dosing was performed at the end of the PCI procedure, and therefore there would have been less $P2Y_{12}$ activity for a number of hours after the administration of the agent with the slower onset of action. Also the majority of patients included were Non-STEMI (NSTEMI) or convalescent STEMI patients undergoing PCI. In the STEMI subgroup (again with a 2:1 convalescent to acute ratio) there was no advantage of the newer agent seen, possibly due to issues with drug absorption and metabolism (14).

Ticagrelor differs from the thienopyridine class of antiplatelet agents (clopidogrel, prasugrel, and ticlopidine) as it does not exist as a prodrug and thus does not require biotransformation by hepatic enzymes before becoming active Also ticagrelor binds reversibly to $P2Y_{12}$ receptors leading to a faster onset and offset than clopidogrel (15). The PLATO trial which compared ticagrelor with clopidogrel, demonstrated an improvement in clinical outcome in the ticagrelor group. (16). Moreover, in the RESPOND study, ticagrelor therapy was associated with uniform and superior platelet inhibition in both previously identified clopidogrel responders and nonresponders, and that inhibition, in turn, was associated with an extremely low prevalence of high on-treatment platelet reactivity (17). Ticagrelor has recently received recommendation in the ESC guideline as a front-line treatment option for STEMI and NSTEMI (18,19)

The synthetic hirudin analogue Bivalirudin is a reversible direct thrombin inhibitor with important effects on the coagulation pathways and also inhibits the activity of circulating and clot bound thrombin, thus also having a partial antiplatelet effect. This agent, when used with dual antiplatelet therapy in patients undergoing PPCI for STEMI, appears to confer a survival benefit compared to heparin, IIb/IIIa receptor antagonism and dual antiplatelet therapy (with aspirin and clopidogrel) (20). This benefit is largely due to a reduction in major bleeding. In the Horizon-AMI trial, however, there was a marked increase in early stent thrombosis in the bivalirudin group (from 1.4% vs 0.3 %).
In view of these positive findings many operators now use bivalirudin routinely (as recommended as a possible treatment by NICE guidance TA 230) but to mitigate against the higher risk of stent thrombosis are giving one of the newer $P2Y_{12}$ antagonists (prasugrel or ticagrelor) which have been shown to lead to lower stent thrombosis rates in other ACS groups (14, 21). Whilst both of these agents have been shown to have a faster onset of action than clopidogrel, there are currently no data on the onset of action required in the setting of PPCI. Ideally a full antiplatelet effect would be evident at the start of the PPCI, whereas in practice dosing of clopidogrel, prasugrel or ticagrelor typically occurs on admission to hospital (possible only 10-20 minutes prior to PPCI).

During PPCI (particularly using bivalirudin rather than i.v. IIb/IIIa antagonists) it is likely therefore that patient's would be advantaged by rapid $P2Y_{12}$ antagonism. Currently there are no data concerning the comparative rapidity of clinical effect of these agents in the setting of an acute STEMI when compared to other groups.

2.2 RATIONALE FOR CURRENT STUDY

It is unlikely that gastro intestinal absorption during a STEMI is equivalent to that of a resting patient. Hypotension and or diversion of splanchnic blood flow is likely to slow drug absorption and the intrinsic increase in platelet activity seen during STEMI (22, 23) may also inhibit the antiplatelet effect of drugs. A previous pharmacokinetic study has shown reduced drug absorption during STEMI (24). In the current study we aim to demonstrate whether prasugrel, or ticagrelor are having a significant clinical effect *during* the primary angioplasty (STEMI patients), and investigate the pharmacodynamic properties of these agents in the setting of STEMI. By also studying patients with unstable angina (UA)/NSTEMI we will investigate whether the condition of STEMI *per se* affects the pharmacodynamics of these agents.

A number of methods have been developed to assess the effect of $P2Y_{12}$ receptor inhibition. Their effect is probably best measured using techniques that specifically measure $P2Y_{12}$ ADP receptor activation, such as vasodilator stimulated phosphoprotein phosphorylation (VASP) and the VerifyNow $P2Y_{12}$ system (Accumetrics, San Diego, CA) (24). We will utilize both of these methods in this study. We will also measure aspirin activity using the VerifyNow aspirin test.

3. STUDY OBJECTIVES

1) To determine the degree and time-course of platelet inhibition in patients admitted with NSTEMI treated with prasugrel within the first 4 hours of loading. These data will be compared with data already collected on STEMI patients treated with prasugrel to determine whether the condition of STEMI per se reduces the efficacy of prasugrel treatment.

2) To demonstrate the degree and time-course of platelet inhibition by ticagrelor given acutely before emergency primary angioplasty for STEMI, during the procedure and in the following 4 hours. This will be compared with a cohort of patients presenting with NSTEMI treated with ticagrelor to determine whether the condition of STEMI per se reduces the efficacy of ticagrelor treatment.

4. STUDY DESIGN

This is a pilot study which is both non-randomised and observational in nature.
We plan on recruiting a total of 45 participants into the study who will comprise of patients admitted to New Cross Hospital suffering from an acute coronary syndrome. The study will complete an on-going project (REC ref no. 13/WM/0025 ethical approval granted from South Birmingham Regional Ethics Committee). Whole blood samples will be taken from participants following a single loading dose of an antiplatelet agent once the diagnosis of an ACS has been made. We anticipate the conclusion of subject recruitment in 3 months from the study start date

4.1 STUDY OUTCOME MEASURES

a) Degree of platelet inhibition measured with VerifyNow ^{TM} rapid platelet function analyser
b) Degree of platelet inhibition measured with VASP flow cytometry
c) Concentration of prasugrel and ticagrelor active metabolite in plasma measured with liquid chromatography with tandem mass spectrometry.

This study is not powered for clinical outcomes; however clinical data will be recorded.

Clinical endpoints to be recorded for each participant will include:

d) Angiographic variables –
- thrombolysis in myocardial infarction (TIMI) flow at end of case,
- slow flow/no reflow

e) ECG variables –
- ST-segment resolution at end of case

f) Safety endpoints –
- Death
- CABG
- TIMI major and minor bleed (see appendix 1 for bleeding criteria)

5. PARTICIPANT ENTRY

5.1 CONSENT

Consent will be a 2 stage process for patients undergoing PPCI for STEMI. Because the drugs given will be as per local and national recommended guidelines, consent will be verbal prior to the PPCI and first 3 blood samples. A shortened patient information sheet will be read to the patient (so as not to delay urgent treatment) and if verbal consent is gained it will be recorded in the clinical notes and the site file consent record. This is in line with NRES guidelines that suggest that patients admitted with STEMI are considered as unconscious patients initially.

Following emergency PCI when the patient is pain free and has been able to rest, a full patient information sheet will be proffered and written consent sought. If the patient does not want to be part of the trial the previous samples will be discarded and the data not used for analysis. Treatment will continue in accordance with clinical guidelines. The patients' participation in the trial will end with the 4hr sample.

For patients with UA/NSTEMI full written consent will be taken after the patient has read the full patient information sheet. Blood tests will be taken at time-points which coincide with STEMI time-points (20 minutes, 60 minutes, and 4 hours post dosage)

Patient Data will be anonymised and stored on RWHT trust fully encrypted computers

5.2 PRE-REGISTRATION EVALUATIONS

All research subjects will be identified from STEMI patients admitted for PPCI at the Heart and Lung Centre in Wolverhampton and also UA/NSTEMI acute coronary syndrome patients admitted to New Cross Hospital, Wolverhampton. Patient groups (below) are carefully selected so that all patients will receive anti-platelet drugs as per current accepted National and local guidelines and as per the manufacturers` datasheets.

1) Group 2: Patients with NSTEMI who are under 75 years of age and greater than 60Kg in weight receiving Prasugrel loading (60mg) however: -
    i. After sample collection patients treated with intracoronary stent placement on the same day as loading will receive prasugrel maintenance dose (10mg per day) as per licensing agreement for prasugrel
    ii. After sample collection patients who are not stented after loading will receive clopidogrel maintenance dose (75mg per day).

2) Group 5: Patients admitted with STEMI receiving ticagrelor loading (180 mg) and then maintenance (90mg bd per day).

3) Group 6: Patients Admitted with NSTEMI receiving ticagrelor loading (180 mg) and then maintenance (90mg bd per day).

We plan to recruit 15 patients into each group.

5.3 INCLUSION CRITERIA

1) Patients presenting with STEMI for PCI (characterized by chest discomfort, and prominent ST-segment elevation)
2) Patients presenting with NSTEMI (characterized by chest discomfort, raised levels of myocardial enzymes and/or ST-segment depression or prominent T wave inversion)
3) Able to give verbal consent (STEMI patient's pre procedure) and/or written consent (STEMI after procedure and NSTEMI patients prior to enrolment).
4) Age>18 years of age
5) Able to take Aspirin and either prasugrel or ticagrelor.
6) Have no concurrent septic or inflammatory illness
7) Thienopyridine naive

5.4 EXCLUSION CRITERIA

1) Be unable to provide verbal and written consent
2) Allergic to aspirin or any of the $P2Y_{12}$ antagonists in the trial
3) Have pre-existing cardiogenic shock
4) Have a concurrent septic or inflammatory disease e.g. rheumatoid arthritis, lupus, pneumonia.
5) Already taking a $P2Y_{12}$ inhibitor
6) Known bleeding diathesis
7) Patients **over** 75 years of age or under 60 kg or those who have had a previous stroke/transient ischaemic attack, will not be eligible for prasugrel but rather ticagrelor.
8) Patients with a history of intracranial haemorrhage will not receive prasugrel or ticagrelor but rather will receive treatment with clopidogrel.

5.5 WITHDRAWAL CRITERIA

Patients who decide to withdraw their consent to participate in the study will have all samples collected up to the time of withdrawal disposed of in appropriate manner. All information collected on the patient for the purpose of the study shall be erased. The withdrawal shall be recorded in the study patient identification log. The treatment of the patient will continue in accordance with clinical guidelines should the participant chose to withdraw or not.

6. ASSESSMENT AND FOLLOW-UP

6.1 Subjects: A total of 45 subjects will be included in the study of which 15 patients will undergo PPCI as part of the treatment for STEMI.

6.2 Sampling

6.2.1 STEMI patients will have a $P2Y_{12}$ inhibitor administered (ticagrelor as per current local and national guidance) following verbal consent. The time of loading dose will be recorded and the patient transferred to the cardiac catheterization suite. After the insertion of the radial or femoral sheath, 15 ml of whole blood will be drawn from this sheath at 20 minutes post dosing (or as close to this time point as practicable) and also at the first balloon inflation time. A further 15 ml will be taken at 60 minutes post dosing (or as close as is practicable). Further sampling on the ward will take place after 4 hours post dosing from an anticubital vein using a 21 gauge needle (see appendix 2 for a flow chart summarizing procedure for these groups).

6.2.2 NSTEMI patients will have a $P2Y_{12}$ inhibitor administered (prasugrel or ticagrelor) following full written consent. The time of loading dose will be carefully recorded. 15 ml of blood will be taken at 20 minutes, 60 minutes and then 4 hours post dosing solely from an anticubital vein using a 21 gauge needle (see appendix 3 for a flow chart summarizing procedure for these groups).

6.3 PHARMACODYNAMIC AND PHARMACOKINETIC ANALYSIS

6.3.1 VerifyNow for aspirin and $P2Y_{12}$ analysis

VerifyNow ™ (Accumetrics, San Diego, California, USA) is a near patient test comprising a turbidimetric based optical detection system that measures platelet aggregation as an increase in light transmittance.

We are already using this system for clinical research in our department and it has FDA approval for the assessment of Aspirin and $P2Y_{12}$ activity. VerifyNow has also been used in the assessment of ticagrelor activity in a number of studies (17, 25, 26). 2 ml of whole blood is transferred into a Greiner Bio-One Vacuette containing 3.2% sodium citrate (Accumetrics, San Diego, California, USA) and inverted carefully, after which the tube is left at room temperature for a minimum of 15 and 30 minutes for both $P2Y_{12}$ and aspirin assays respectively. The tube is then loaded into a specific assay device and analysed as per the manufacturers' instructions (Accumetrics). The system is calibrated daily electronically and full quality control is performed with each batch of assay devices. This system uses a similar principle to formal platelet aggregometry. Arachidonic acid is used in the aspirin assay, ADP in the $P2Y_{12}$ assay. Aspirin will already have been given in the

ambulance and its antiplatelet effect will be assessed at 20 minutes and 60 minutes after the $P2Y_{12}$ inhibitor loading dose

Results are shown as aspirin response units (ARU) and $P2Y_{12}$ reaction units (PRU) for aspirin and $P2Y_{12}$ assays respectively.

6.3.2 VASP flow cytometry

VASP, an intracellular actin regulatory protein, is a substrate of both cyclic adenosine monophosphate (cAMP)- and cyclic guanosine monophosphate (cGMP)-dependent protein kinases (27). Dephosphorylation of VASP follows $P2Y_{12}$ stimulation. Conversely, inhibition of the $P2Y_{12}$ receptor by clopidogrel and stimulation of prostaglandin E_1-activated adenylyl cyclase induce phosphorylation of VASP by cAMP-dependent protein kinases. Thus levels of VASP phosphorylation/dephosphorylation reflect $P2Y_{12}$ inhibition/activation.

Despite using a commercially available assay (Biocytex, Asnieres, France), VASP flow cytometry is primarily a research based assessment owing to the complexity of the procedure and the requirement for significant technical expertise. It is seen by some as a gold standard test for $P2Y_{12}$ inhibition.

Whole blood transferred to tubes containing 3.2% sodium citrate is incubated with PGE1 or PGE1 and ADP for 10 minutes then fixed with paraformaldehyde, then platelets are permeabilized with non – ionic detergent. The cells are than labelled with a monoclonal antibody against 239-phosphorylated VASP and then a secondary fluoroscein isothicyanate-conjurgated polyclonal antibody. Analysis will be performed on a Becton Dickinson (Plymouth, UK) FACS Calibur flow cytometer, and 10,000 platelets will be gated per assessment as per the manufacturer's instructions. Results are expressed as percentage platelet reactivity index (% PRI). VASP flow cytometry will be performed within 48 hours of sampling.

VASP Flow cytometric analysis measures activity at the $P2Y_{12}$ receptor very effectively and thereby indirectly the activity of $P2Y_{12}$ inhibitor and has been shown to be of value in a number of clinical trials including one carried out by our own research group (28, 29).

6.3.3 Liquid chromatography with tandem mass spectrometry.

We will be using this technique to quantify the active metabolites of prasugrel, and ticagrelor. The active metabolite of prasugrel are not stable in blood, therefore 3'-methoxy-phenacylbromide (an alkylating reagent) will be added within 30 seconds of sampling to whole blood collected in EDTA tubes to derivatize and stabilize the metabolites. Derivatized blood samples will be centrifuged at 1500g for 10 minutes. The plasma will be extracted and frozen at -80C until analysis.

Blood samples for ticagrelor active metabolite quantification will be collected in lithium heparin tubes. The active metabolites of ticagrelor are stable in blood, thus require no

stabilization. The blood samples will undergo the same processing and storage protocol as highlighted above.

The derivatized and ticagrelor plasma samples then undergo solid phase extraction and are then separated using liquid chromatography and quantified using mass spectrometry. The primary pharmacokinetic parameters (i.e. the active metabolites of prasugrel, and ticagrelor) are calculated using the maximum observed concentration (C_{max}) and area under the plasma concentration curve (AUC). This technique has already been developed and validated used previously to detect the active metabolite of either prasugrel and ticagrelor in a number of publicized studies (30-34).

To summarize, each sample will be used for

1) $P2Y_{12}$ inhibition estimation using the near patient Accumetrics VerifyNow test

2) $P2Y_{12}$ inhibition will also be estimated by Flow Cytometry using VASP phosphorylation test.

3) Aspirin effect using near patient Accumetrics VerifyNow test.

4) The active metabolites of prasugrel and ticagrelor will be measured using liquid chromatography with mass spectrometry.

Time	Pre Dosing	20 Min post dosing	Balloon Time (PPCI)	60 min post dosing	4 hrs post dosing
Procedure	Consent	15 ml blood	15 ml blood	15 ml blood	15 ml blood
VN Aspirin		x	X	x	
VN $P2Y_{12}$		x	X	x	X
VASP Phosphorylation		x	X	x	X
Estimation of active metabolite		x	X	x	X

7. STATISTICS AND DATA ANALYSIS

All statistical analysis will be performed in collaboration with Professor Nevill, Professor of biomedical statistics at University of Wolverhampton.
Continuous variables will be compared using parametric and non-parametric tests as appropriate. Dichotomous variables will be compared by Fishers exact test. Logistic regression analysis will be used to predict the significant independent predictors of poor outcome post angiography.

8. END OF STUDY

The study will come to an end when the participant recruitment target has been met, all samples have been analysed and the data has been scrutinised, and finally an article

summarising the study written and submitted to a peer reviewed journal for consideration of publication.

9. ADVERSE EVENTS

9.1 DEFINITIONS

9.1.1 Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

9.1.2 Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or affect that:

- **Results in death**
- **Is life-threatening** – *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 which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

9.2 REPORTING PROCEDURES

All adverse will be reported. Depending on the nature of the event the reporting procedures below will be followed. Any questions concerning adverse event reporting will be directed to the Chief Investigator in the first instance.

9.2.1 Non serious AEs

All such events, whether expected or not, will be recorded.

9.2.2 Serious AEs

An SAE form will be completed and faxed to the Chief Investigator within 24 hours. However hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

Information about all serious adverse events will be collected and recorded on the Serious Adverse Event Report Form and faxed to the R&D Directorate at New Cross Hospital within 24 hours of learning of its occurrence.

All serious adverse events will be reported to the appropriate regulatory and ethical authorities.

Contact details for reporting SAEs
Fax: 01902 695646, attention Dr James Cotton
Please send SAE forms to: Dr James Cotton
Tel: 01902 694200 (Mon to Fri 09.00 – 17.00)

10. REGULATORY ISSUES

10.1 ETHICS APPROVAL

The Chief Investigator has previously obtained approval from the South Birmingham Research Ethics Committee (REC ref. 13/WM/0025).

10.2 CONSENT

Fully informed consent will be taken from the patient.

10.3 CONFIDENTIALITY

Blood samples will anonymised with members of the research team at New Cross Hospital having access to the patient's details. All data collected on the patient will be stored in an anonymised fashion on and stored on password protected computer at New Cross Hospital. Data and all appropriate documentation will be stored for a minimum of 5 years after the completion of the study, including the follow-up period.

10.4 INDEMNITY

Standard NHS Indemnity is in place for this study only.

10.5 SPONSOR

The Royal Wolverhampton NHS Trust has agreed to act as Sponsor for this Study.

10.6 FUNDING

This study has been part funded by the United Kingdom Clinical Pharmacy Association.

10.7 AUDITS AND INSPECTIONS

The study will be audited and monitored by the sponsor's monitoring team.

11. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated by Dr Vincent Amoah.

Trial Management Committee:

Due to the nature of the study in that this is not a randomised controlled trial, and the drugs being used for the study are licenced for this indication and are in use, a Data Monitoring Committee will not be required. In this study the Trial Management Committee will protect patient safety. If adverse events of a particularly serious type are occurring frequently, then the TMC would have to strongly consider termination of the study. This evaluation will be made in consideration of risk/benefit.

12. PUBLICATION POLICY

We intend to submit an article detailing the results of the study to a peer review journal for consideration of publication.

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APPENDICES:

Appendix 1 – Criteria of major and minor TIMI bleeding (35-37)

1. Major

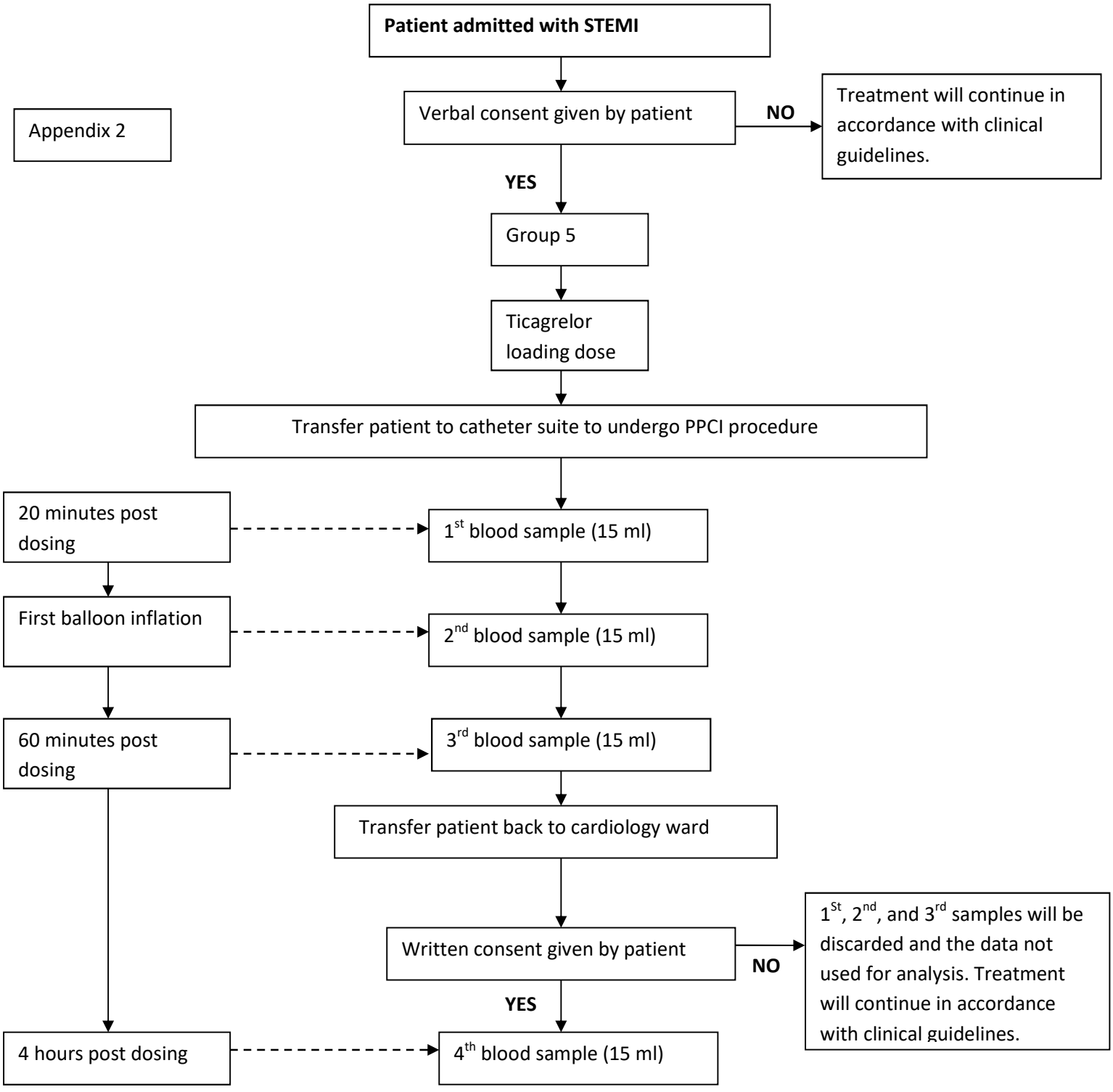
- Any intracranial bleeding (excluding microhaemorrhages <10 mm evident only on gradient-echo MRI)
- Clinically overt signs of hemorrhage associated with a drop in hemoglobin of ≥5 g/dL or a ≥15% absolute decrease in haematocrit
- Fatal bleeding (bleeding that directly results in death within 7 days)

2. Minor

- Clinically overt (including imaging), resulting in hemoglobin drop of 3 to <5 g/dL or ≥10% decrease in haematocrit
- No observed blood loss: ≥4 g/dL decrease in the haemoglobin concentration or ≥12% decrease in haematocrit
- Any overt sign of hemorrhage that meets one of the following criteria and does not meet criteria for a major or minor bleeding event, as defined above:

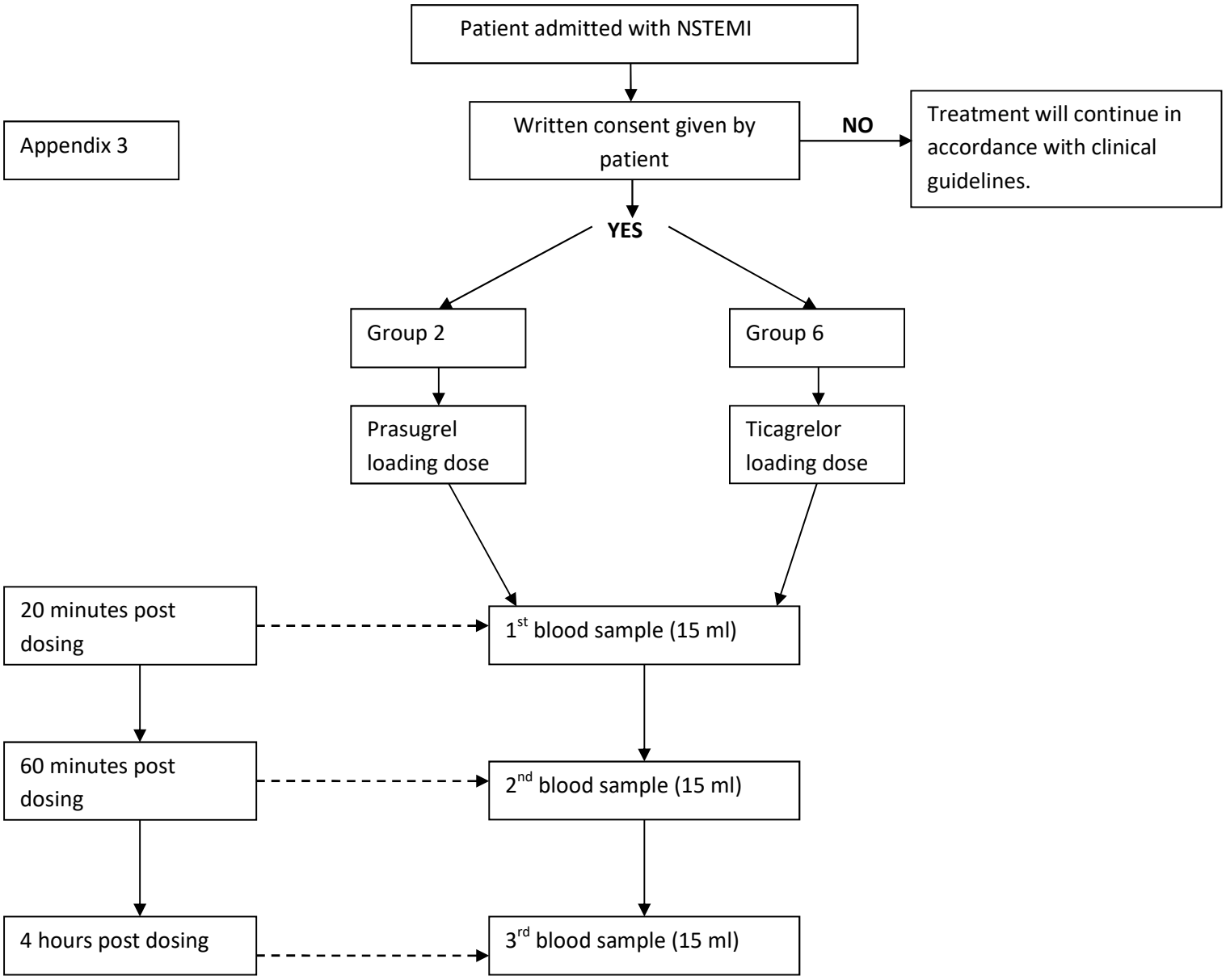
    - Requiring intervention (medical practitioner-guided medical or surgical treatment to stop or treat bleeding, including temporarily or permanently discontinuing or changing the dose of a medication or study drug)
    - Leading to or prolonging hospitalization
    - Prompting evaluation (leading to an unscheduled visit to a healthcare professional and diagnostic testing, either laboratory or imaging)

Appendix 2



Each blood sample taken will be subjected to analysis using VerifyNow, Flow Cytometry using VASP phosphorylation, and liquid chromatography with mass spectrometry.

Appendix 3



Each blood sample taken will be subjected to analysis using VerifyNow, Flow Cytometry using VASP phosphorylation, and liquid chromatography with mass spectrometry.