# - GRAFITE -

(**G**lycoprotein VI **R**eceptor in **A**trial **FI**brillation and **T**hrombo**E**mbolism)

*Study Protocol Version 2.0*
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REC (16/EE/0436)


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## 1. INTRODUCTION AND STUDY BACKGROUND

Platelets are the cells crucial for arresting bleeding at sites of tissue injury. As well as this, they play a key role in the formation of blood clots (thrombosis) within blood vessels which can lead to heart attacks, stroke and peripheral vascular disease.

The GRAFITE (Glycoprotein six Receptor in Atrial FIbrillation and ThromboEmbolism) study will investigate the involvement of platelets in unwanted blood clot formation in two specific conditions in patients admitted to hospital – atrial fibrillation (AF) and venous thromboembolic disease (VTE). The research team will investigate any genetic variances that cause blood clot formation, as well as looking at the platelet receptor glycoprotein VI (GPVI) dimer and its role in clot formation in these conditions.

Atrial Fibrillation is the most common cardiac rhythm disturbance encountered in clinical practice and the risk of developing AF increases with age.[1] Not only does having risk factors that lead to heart attack and stroke increase your risk of developing AF, having AF increases your risk of having a stroke by 2 to 7 times.[2,3] This is caused by the 'fibrillating' movement of the atrial heart chambers, causing a blood clot to build-up in the heart, which can then migrate to the brain causing a stroke. The prevention of stroke from atrial fibrillation is a cause of ongoing research, as healthcare expenditure is continuing to rise as a result of this condition.

Similar blood clots can also develop in blood vessels such as veins, where they are called venous thromboemboli. This includes conditions such as deep vein thrombosis. VTE can be common in patients admitted to hospital due to their comorbidities and relative immobilisation compared to normal, due to illness. It is believed that the clot formation in atrial fibrillation and venous thromboembolism are similar, but the crucial involvement of platelets, especially glycoprotein VI, is not fully understood.

Atherosclerosis is the process that is responsible for most heart attacks and strokes. This is due to the development of cholesterol-rich plaques blood vessels due to the culmination of risk factors such as age, smoking, high blood pressure and diabetes. When these plaques rupture, collagen is exposed and a blood clot starts to form which can then migrate to distant places causing heart attacks and stroke. There is currently a mass of evidence which points to the platelet surface receptor, glycoprotein VI, as the main receptor involved in the early stages of blood clot formation that lead to strokes and heart attacks due to its interaction with exposed collagen at these sites of plaque rupture.[4–7]

What is not known, is its role in clot formation in AF and VTE where the platelet clumping and blood clot formation is independent of plaque rupture and binding to collagen. There is recent emerging evidence that fibrin, a compound which acts to stabilise a blood clot via different mechanisms interacts with GPVI and activates platelets, although very little is known about this process at present.[8,9]

The management of both these conditions lies in anticoagulation, thinning the blood to reduce the risk of the blood clot migrating to other places like the brain or the lungs, and there is overwhelming evidence that antiplatelets (used to treat strokes and heart attacks) are not effective in these conditions.[10,11]

The GRAFITE study will aim to take blood samples from patients (once, during their admission) with these conditions, admitted to Cambridge University Hospital under a medical team. The samples will then be analysed for their platelet function, specific studies on the role of glycoprotein VI in clot formation and analysing for genetic variants which are associated with atrial fibrillation, venous thromboembolism, stroke or related phenotypes.

With our research we aim to look into the role GPVI plays in blood clot formation in these conditions via its interaction with fibrin and other compounds that are involved in clotting. As well as this, the blood samples will allow us to look at any common themes in patients' genes which may increase their susceptibility to atrial fibrillation, venous thromboembolism or stroke.

With the results of this study we hope to be one step closer to developing drugs that are highly specific to platelet receptors that are involved in clot formation. Our priority is prevention of stroke from AF as well as safer treatment of VTE by alleviating some of the risks that come with taking the existing drug treatments.

## 2. STUDY OBJECTIVES

This research project would investigate the genetic and biochemical mechanisms that put patients with AF at risk of stroke, as well as development of VTE, with particular focus on the glycoprotein VI platelet receptor. Data obtained will be compared to controls who have been recruited under separate ethics for the studies entitled "Genetic analysis of platelets in healthy individuals" (REC reference 10/H0304/65) and "Genes and platelets in stroke (GYPSIE) study" who are investigating the GPVI dimer after different aetiologies of stroke (REC reference 14/EE/1062).

**Hypothesis:** Glycoprotein VI dimer levels are increased in patients with AF and VTE on the platelet surface, with evidence of increased platelet activation. GPVI interacts with fibrin(ogen) to stabilize growing thrombus.

### *2.1 Primary objectives*

This research project will investigate the genetic and biochemical mechanisms that make platelets stick together and form a blood clot, with particular focus on the glycoprotein VI (GPVI) dimer which is a platelet surface receptor that helps platelets clump together.

The primary objective:

1. Measuring platelet glycoprotein VI dimer levels and platelet activation (as measured by P-selectin exposure, response to ADP, fibrinogen, CRP, TRAP binding) in patients admitted to hospital with atrial fibrillation and venous thromboembolism by flow cytometry. The results will be compared to subjects from "Genetic analysis of platelets in healthy individuals" (REC reference 10/H0304/65) and “Genes and platelets in stroke (GYPSIE) study” who are investigating the GPVI dimer after different aetiologies of stroke (REC reference 14/EE/1062) studies.

### *2.2 Secondary objectives*

The secondary objectives would be to investigate whether:

1. Laboratory investigations and adhesion studies to investigate the relationship between glycoprotein VI and fibrin, in thrombus formation and growth.

2. Investigate the correlation between glycoprotein VI dimer levels on flow cytometry and known common blood biomarkers for atrial fibrillation, coagulation and stroke.

3. Investigate the correlation between glycoprotein VI dimer levels and estimated risk of stroke (as stratified by the $CHADS_2$-$VASC_2$ score which is commonly used in clinical practice).

4. Investigate the genetic basis for atrial fibrillation and venous thromboembolism and a therefore look for a genetic basis for possible pre-disposition to stroke.

## 3. METHODOLOGY

We will look at the genetic and biochemical mechanisms of blood clot formation in atrial fibrillation (AF) patients and those who are diagnosed with venous thromboembolic disease (VTE).

This will be investigated by laboratory testing on blood samples (one single blood draw) collected from patients who have atrial fibrillation (either a new diagnosis or known) or venous thromboembolic disease, who are admitted to hospital.

The stepwise methodology is illustrated in Figure 1.

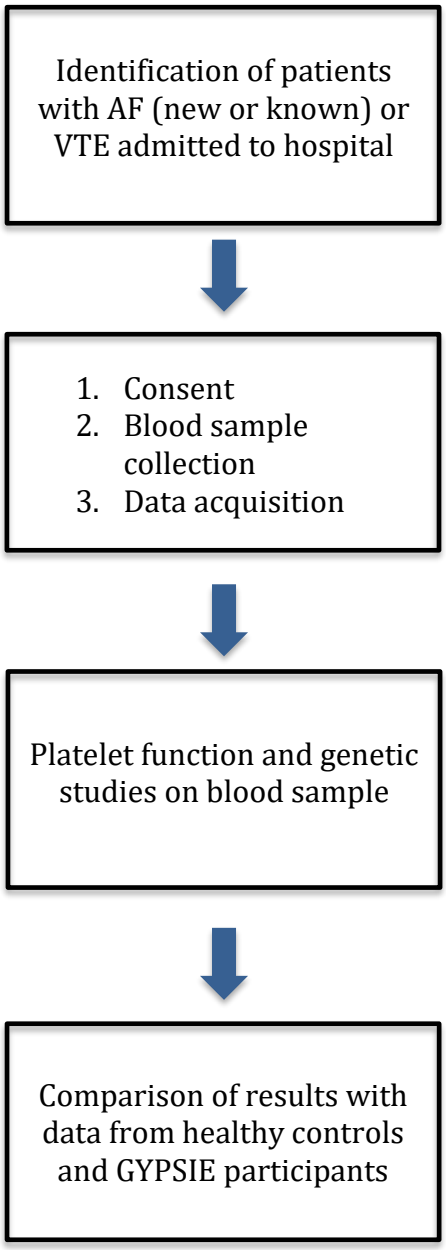


*Figure 1. Study design*

### *3.1 Inclusion Criteria*

Cambridge University Hospital admits around 1300 patients a month under general medicine via the emergency department. We aim to include patients with atrial fibrillation who are admitted under a medical team with AF as a main diagnosis or a comorbidity. Patients with a new diagnosis of AF would be diagnosed by 12-lead ECG which is routinely carried out for every patient whilst

they are in the emergency department. Paroxysmal (intermittent) AF carries the same risk of stroke as persistent AF, and therefore patients who have a history of AF but were in normal sinus rhythm at the time of admission would be included.

Venous thromboembolic disease is common in both hospital and community settings due to various risk factors. Patients with suspected VTE attend the emergency department, where they are usually treated presumptively with blood thinning medication, until a scan happens via the ambulatory care pathway the following day. We would aim to recruit patients attending the ambulatory care department, once they have a scan confirming VTE.

The inclusion criteria:

1) Patients with a new diagnosis of AF and those with persistent or paroxysmal AF, whether they are treated on anticoagulation or not.
2) Patients with confirmed venous thromboembolic disease, whether they are treated on anticoagulation or not.
3) Attending Cambridge University Hospitals.

### *3.2 Exclusion Criteria*

The following patients would be excluded from the study.

1) No clear confirmation of the presence of atrial fibrillation in medical notes or ECG (for AF patients)

   or

2) No confirmed venous thromboembolic disease on any imaging modality (only for those with suspected VTE)

3) Not admitted under a medical team to Cambridge University Hospital or attending the ambulatory care unit.
4) Age less than 18 years
5) Pregnancy
6) Active and known malignancy
7) Known platelet disorder
8) Haemoglobin <95g/L at the time of blood sampling
9) Known HIV/AIDS
10) Known hepatitis B or hepatitis C infection
11) Admitted with a transient ischaemic attack or stroke – ischaemic or

haemorrhagic.

12) Patients who lack capacity

### *3.3 Sample Size*

The current results from the GYPSIE project measures the mean fluorescence intensity (MFI) of the GPVI dimer in patients with stroke (mean MFI= 1.65) compared to healthy normal (mean MFI =1.5). Power calculations using these control parameters suggest that we can resolve 10% differences in GPVI dimer MFI between two donor populations (at $p<0.05$) with 80% power using a group size of less than 60

Therefore, we plan to recruit 60 new/not on anticoagulation and 60 known/on anticoagulation atrial fibrillation patients as well as 60 VTE patients.

## 4. STUDY PROCEDURES

### *4.1 Participant recruitment*

**The method of recruitment for AF and VTE patients will differ,** but the consenting process, blood draw and laboratory analysis will remain largely the same.

The study will include patients attending Cambridge University Hospital with atrial fibrillation or venous thromboembolic disease. They will be under the care of various general medical teams but all potential participants will fall under the umbrella of divisions C + D at Cambridge University Hospital. Prior authorisation by the directors of divisions C + D will be obtained before patient recruitment takes place.

**DIVISION C:** ACUTE MEDICINE; INFLAMMATION/INFECTION; ~~TRANSPLANT~~
**DIVISION D:** NEUROSCIENCE; ~~ENT/ HEAD AND NECK/ PLASTICS;~~ CARDIOVASCULAR-METABOLIC

Participants will be recruited from acute medicine, inflammation/infection, neuroscience, cardiovascular-metabolic cohorts. Patients from transplant, ENT and plastics will be excluded as they are surgical specialties.

The research team will ensure that we introduce ourselves to staff members on the ward, including but not restricted to the medical and nursing teams before patients are approached. If the clinical team feel that study participation is not in the patient's best interests, or recruitment should be delayed, the research team will not approach the patient for participation.

It will be made clear to the patient that the blood sample obtained will be purely for research purposes and will not impact on their clinical care.

#### *4.1.1 Recruiting patients with atrial fibrillation*

The details of patients admitted from the previous day is discussed at morning report, a meeting that would be routinely attended by members of the research team including stroke nurses. Patient comorbidities (including AF and VTE) would be discussed at this meeting. Medical morning report will be the main source of identification of patients admitted with atrial fibrillation, attendance at this meeting is routine for research teams in stroke.

If AF has been identified in a patient admitted in the previous day, it would be made clear at morning report. The research team will ask if the direct care team would approach the patient and ask their consent for the research team to see if they fit the inclusion criteria and if the individual would be willing to consider participation.

If the individual fits the criteria, the research team would approach the individual in order to provide more information about the study and allow time to consider participation as outlined below.

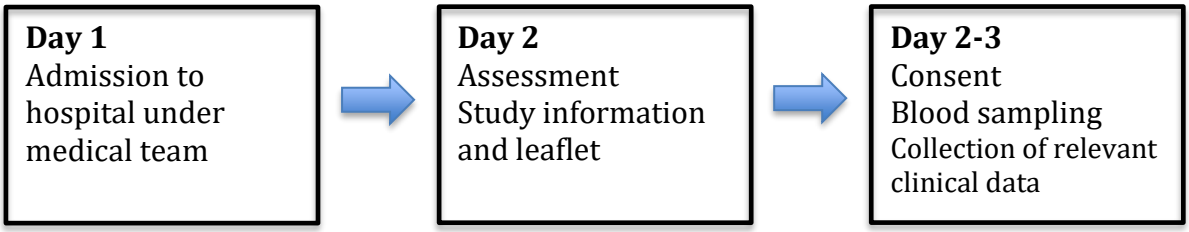


*Figure 2. Assessment will typically take place on day 2 of admission. Consent will be gained after study information provided to patient they have had a chance to consider participation – typically 24h later.*

#### *4.1.2 Recruiting patients with venous thromboembolic disease*

Patients with venous thromboembolism usually attend the 'ambulatory care' clinic on ward EAU3 at Cambridge University Hospital. This can be a direct admission, or more commonly, they attend the emergency department the day before with suspected VTE, are started on treatment, and asked to attend the ambulatory care clinic for a scan to confirm VTE or not.

If confirmed with VTE, the direct care team can notify the research team that there is a potential participant. They direct care team would have a brief discussion with the patient with regards to involvement and disclosing their details to the research team prior to this. Further detailed discussions about participation and the study would be carried out by the research team. Fully informed consent will also be carried out by the research team.

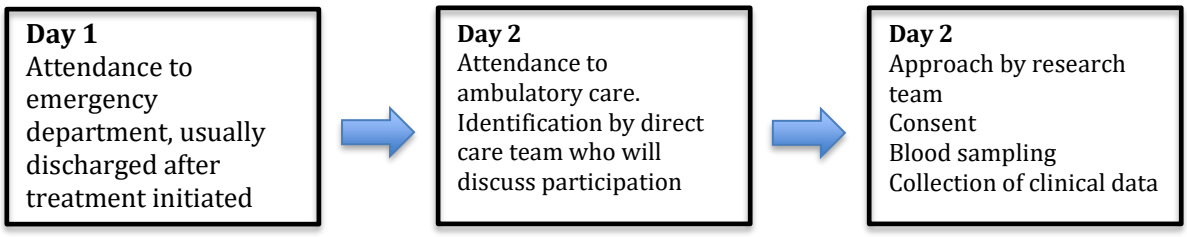


*Figure 3. Assessment will typically take place in the ambulatory care department. Patients who are returning the next day or soon after can have the patient information leaflet to take home and consent and allow blood draw at their next visit.*

### *4.3 Consent*

The study design will only include patients who are able to consent for themselves. A 'participation information leaflet' which would include the full details of the study would be provided to those patients presenting in hospital with atrial fibrillation or venous thromboembolism early in their admission.

This would usually be day 2 of admission, once the acute clinical treatment of their condition has been commenced. This would be done by an appointed research nurse or doctor familiar with the study.

The patient would then be visited 24 hours later (for AF patients) or when it is clinically appropriate or convenient to the patient (for VTE patients who are likely to be discharged the same day) where there will be an opportunity to answer any pertinent questions that they might have.

If they agree to participate in the study, informed consent would be obtained in writing and a blood sample would be taken at that point.

### *4.4 Blood sampling*

No more than 50ml of blood would be taken from each patient using normal phlebotomy methods e.g. direct venepuncture or at the time of cannulation. We would aim to time these at the same time that their usual blood samples are taken to maximise patient comfort and safety.

### *4.5 Location of obtaining blood samples*

Blood samples will be taken at Cambridge University Hospital, usually on a ward or ambulatory care department. If a patient consents to participation after they are discharged from the study, or there is no possibility of obtaining their blood

sample after consent is obtained, but before discharge, they would be invited to attend at a time of their convenience to have their blood sample taken. Therefore, other locations of blood draw would include suitable locations within the Addenbrooke's campus including the NHS Blood and Transplant blood centre, clinical neurosciences R3 etc) depending on what suits the participant.

### *4.6 Clinical data*

After consent is obtained from the patient, further details would be gained from the patient's medical notes. This would include:

1) Basic demographics data
2) Medication details
3) Clinical details such as admission diagnosis, medical/surgical history, AF history (if appropriate) and comorbidities
4) Smoking and alcohol history
5) Information about blood results (biochemistry, haematology, immunology, pathology)
6) Any imaging (CT head, echocardiogram, ultrasound scan)

The blood samples would be accompanied by basic demographic details, medication history and clinical details to start with. The other details e.g. bloods results and imaging, can be gained at a later date and added into a secure database.

### *4.7 End of study definition*

The definition of the end of the study is the last patient that is recruited. There is no follow up related to the study, although patients will receive their usual follow up care related to their admission.

### *4.8 Participant withdrawal*

Participant clinical history, test results and other relevant clinical information will be recorded, maintained and archived in research dedicated databases (hereafter called Project Database) and participant specific notes (hereafter called Research Records).

Study participants are free to withdraw their consent at any time without giving any reasons by notifying the study team by email, phone or mail. If consent is withdrawn, no additional data will be added but already obtained results may be used. Because of mirroring of the computerised Project Databases it is not possible to remove all unique person identification number (UPIN) and unique sample identification number (USIN) related data from the databases, but a flag will be applied to the data indicating that no further data will be added to the records and the data is blocked for future use.

It will not be possible to remove results of any tests already obtained from from the Project Database or Research Records. The central stocks of blood samples or derivatives will be removed from the central research repository within 10 working days after notification of withdrawal has been received. It will, however, not be possible to remove samples which already have been distributed to research laboratories.

# 4 SAMPLE PROCESSING AND ANALYSIS

## *5.1 Analysis of blood samples*

Genetic and platelet function tests will be performed on the obtained blood sample, and results will be saved in secure research databases.

The blood tests can only be performed on fresh blood samples will be performed on the same day as the blood draw (within 6 hours of sample collection).

**Full blood count analysis (FBC).** A routine FBC analysis will be performed on all blood samples received within the allocated time as the results obtained from older samples are less reliable. We will also measure other common biochemical markers of blood clotting and inflammation (**d-dimer and high sensitivity CRP)** as well as BNP **(brain natriuretic peptide)** in patients to see if levels of these biomarkers correlate to levels of GPVI dimer.

**Platelet and other blood cell function tests.** Functional tests of blood cells are only valid if produced on fresh samples because platelet and other cell function can diminish with time. The function of platelets and other blood cells will be measured using the most up-to-date test platforms available.

One of the key questions addressed in this study is what happens to patient's platelets when they have atrial fibrillation and venous thromboembolism along with other related comorbidities such as hypertension and diabetes. Platelets and different types of white blood cells are a key component of the stroke process, and drive the process that ultimately leads to the formation of a clot that causes the stroke. Therefore, any characterization of size, number, biochemical make-up or function of platelets and white cells could bring new information about the biological processes underlying thrombus formation.

We will be using several platforms to investigate the function of blood cells such as flow cytometry and flow adhesion but alternative platforms will be applied during the study.

**Glycoprotein VI (GPVI) dimer measurement**

GPVI is a receptor protein on the platelet surface which when active (GPVI dimer) binds to the damaged blood vessels and help clump platelets together creating a thrombus. Current research indicates that the turbulent flow created within the atria of the heart due to AF will cause sufficient injury to the vessel wall which would drive thrombus formation.

The GPVI dimer levels will be measured using antibodies to determine if they differ in AF patients who have not had a stroke compared to controls without AF (which are collected under separate ethics and study), and stroke patients (collected under separate ethics and study). Measurement of GPVI-dimer in AF patients will determine if high levels of GPVI-dimer are a risk to developing stroke and may help elucidate a secondary role in predicting stroke risk and severity.

To standardize the measurements of each patient's GPVI level, a calibration curve will be established using a standard protein of known concentration. Further, measurements of expressions of other platelet membrane proteins may be included in the tests on the participant's samples, since platelet membrane proteins, including GPVI, may vary widely in the population.

**GPVI activation with fibrin**

Turbulant flow within the atria of the heart causes disruption in the vessel wall within these chambers. Platelets then attach rapidly to these disrupted areas – a critical step in thrombosis. In patients with atrial fibrillation, clot formation is believed to be due to the stasis of blood due to the impaired contractility of the atria due to the fibrillating movement. Platelets interact with the procoagulant factors released in this environment to aid in clot formation, however, the full extent of the involvement of platelets are yet to be determined.

Recent research suggests that GPVI is activated on platelet surfaces by fibrin, a protein which is involved in the growth of blood clots, and clot stability when polymerized. This suggests an alternative method of thrombus formation using the platelet receptor GPVI in addition to what is known already. Our study will attempt to quantify whether GPVI does bind to fibrin in patients who have atrial fibrillation using methods such as flow cytometry and study thrombus propagation and inhibition via GPVI blockade.

**Preservation of nucleic acid**

New tests have become available very recently that can be used to test the interplay between nature and nurture. The DNA code is passed on through the generation from parents to child. However, the way the code is interpreted is influenced by the interplay between our environment, diet, microbiota and other cues and the genes which came from our parents. These effects on how the environment influences the way the DNA code is interpreted can now be accurately measured on small number of fresh blood cells. We may process and purify fresh blood cells in such a way that these "epigenome" tests can be performed later.

**Genetic tests**

One of the aims of the study is to identify genetic variants which are associated with atrial fibrillation, venous thromboembolism, stroke or related phenotypes. This will be done using interrogation of DNA sequence variation e.g. by

genotyping tests and sequencing of the whole genome or a part of it.

At the start of the study genotyping of study samples will be performed using the Affymetrix UK Biobank Axiom Array, which is a commercially available genotyping array with over 800,000 genetic markers. This provides coverage of single genetics variants spread across the genome (so called Single Nucleotide Polymorphisms or SNPs) and allows for testing between correlations of genes and disease status, clinical sub-phenotypes and biochemical phenotypes such as gene and protein expression level and the activity of blood cells, including platelets.

As other more comprehensive genotyping assays are likely to become available during the study duration, we aim to collect genetic data using the most up-to-date platform. We expect to move on to other more comprehensive genotyping platforms as they become available and cost effective. More comprehensive genotyping or sequencing technologies will be implemented when they become feasible in the context of the study.

**Gene expression**

As all cells with a nucleus contain the same DNA, it is differences in expression of these genes that make cells different. Differences in gene expression for specific genes when comparing cases and controls could be indicative of an association between the gene/protein and disease. Gene expression profiling may be performed using microarray expression arrays. However, as with the quick progress of sequencing technology, whole transcriptome analysis by sequencing RNA are becoming financially feasible. The benefits of sequence based expression analysis is that the entire transcriptome can be assayed, whereas microarray methods are limited to assaying the transcripts for which baits are present on the microarrays. As with the genetic tests, we foresee a move to more comprehensive assays as more comprehensive assays become cheaper in the coming years.

**Metabolomics**

High throughput analysis of the chemical composition of the blood plasma or other blood components produces measurements of thousands of chemical compounds giving an instantaneous snapshot of the physiology of that sample. Such analysis will be performed using standard methodology such as NMR spectroscopy or mass spectrometry.

Other “omics” platforms then the “metabolomics one” have been developed and researchers at Cambridge University Hospitals always strive to apply the best tests to the archived biological samples from research study participants.

Increasingly over the last 5 years such tests have been centralised and are being applied to all samples of a certain study. Most “omics” tests are based on massive parallel measurement of hundreds, thousands or even tens or hundreds of thousands of analytes. If a new “omics” test platform has proven to be effective in

the discovery of biomarkers or genetic markers, then often the test will be applied to the entire collection of existing samples from several studies to determine the value of a certain set of markers between and across diseases. Stored and archived samples of plasma, serum or nucleic acid collected for this study may be therefore applied to new "omics" platforms which are currently being evaluated or are coming to the market during the duration of this study.

### *5.2 Sample storage and sharing*

Blood samples used for laboratory testing of GPVI dimer measurements and flow studies of GPVI interaction will be used the day that the sample is obtained and once the laboratory measurements are done, the samples are appropriately destroyed on the same day.

The genetic analysis of blood samples will take place in the Department of Haematology research laboratories located at the NHS Blood and Transplant building. This analysis will include extraction, storage and analysis of blood components such has DNA, RNA, proteins, cells, carbohydrates and lipids as well as extraction of blood components and biomarkers.

In order for the most up to date available platform of analysis to be used, the blood samples and components/derivatives obtained may be extracted and stored in a secure repository for later use. The study participants will agree to storage of their donated samples on the Informed Consent Form. All biological samples will be anonymized and only linked to personal details (held separately) by a study number and stored for the duration of the study, after which they will be destroyed.

### *5.3 Data analysis*

Data obtained from analyses of blood samples collected for this study will be compared to results obtained from healthy controls collected under separate, already existing, ethical approval (Genetic Analysis of Platelets in Healthy Individuals - REC reference 10/H0304/65) and "Genes and platelets in stroke (GYPSIE) study" which is investigating the GPVI dimer after stroke (REC reference 14/EE/1062).

The relevant biomarkers (hs-CRP, d-dimer and BNP) measurements will be carried out at the Cambridge University Hospital pathology laboratories.

## 6 ETHICS

The study will be conducted according to the protocol and in the spirit of ICH GCP, the Declaration of Helsinki and all applicable regulatory requirements.

The following ethical issues have been considered:

**Study design and risk conferred by study procedures**

Participants will be asked to provide blood samples for investigations that are currently still considered to be research tools and as such might not provide us with an insight into their condition. However, the risks of providing a blood sample is relatively small compared to the gain that may be achieved for future generations of patients with these debilitating diseases. The blood draw will be timed as much as possible, with blood taken at the time of other routine blood samples on the ward to minimise patient distress.

In some cases, a separate venepuncture may be needed, e.g. in the case that routine bloods have already been collected by the time consent is obtained and no further blood tests are planned or where consent has been obtained after discharge from hospital.

However, due to the small amount of blood needed and the routine nature of a venepuncture, this will not present any additional risks to the patients due to their disease state and will not alter the treatment for their admission condition in anyway.

The blood will be drawn by an experienced member of the study team, and the risks and discomfort of a blood draw are minor. If requested, the site of venepuncture can be numbed using an anaesthetic agent to reduce discomfort.

**Study recruitment and informed consent**

Patients will be identified by the study team after admission to hospital under a general medical team. As only patients with capacity to consent will be recruited, they will be provided with participant information leaflet when they are seen by the study team, and given an explanation of the information contained within the leaflet. The study team will be available to answer any questions patients may have before signing the informed consent.

Patients for this study cannot be pre-identified or invited to participate but will be recruited as they present at hospital. Patients will only be asked to provide informed consent after a discussion regarding the study information with a member of the study recruitment team.

We plan to put information posters up on the wards of division C+D and the ambulatory care department so that the clinical team can notify us of any suitable patients. For example, those have developed atrial fibrillation or VTE whilst in hospital.

These posters will not be in public areas e.g. waiting rooms but in doctors and nurses offices to act as a reminder to contact the research team if a suitable patient is met.

Consent forms will be kept in a secure location on the Cambridge University Hospitals site with access only with staff swipe cards or keypads.

**Confidentiality**

Participant clinical history, test results and other relevant clinical information will be recorded, maintained and archived in research dedicated databases (hereafter called Project Database) and participant specific notes (hereafter called Research Records).

All samples will be handled in a linked anonymised fashion, meaning that no personal details such as surname, first name, date of birth or address will appear on stored samples and Research Records.

Personal and contact details (surname, first names, date of birth, address, phone numbers, same data from relatives, etc.) are maintained in a separate secure database on NHS computers at Cambridge University Hospitals or computers at the University of Cambridge, Department of Haematology, located in the NHS Blood & Transplant (NHSBT) building with swipe card door access, and keypad locked offices.

Study participants will receive a unique sample identification number (USIN) and a unique person identification number (UPIN) which will be linked to the USIN. UPIN and USIN and linked study information will be maintained in the Project Database that is in the computer environment of the University of Cambridge.

Link tables between the UPIN and personal details will only be maintained in the safe database in the NHS Blood and Transplant (NHSBT) computational environment. The link table will only be accessible to a limited number of senior staff members on the clinical or study coordination team.

Use of the link table is limited to initiate and maintain contacts with participants and access is restricted by unique username and strong passwords which cannot be shared.

Records of all verbal contacts with study participants will be maintained on the Project Database and/or in Research Records.

**Risk and benefit**

The new post-genomics test platforms that are now applied in research generate very dense and large data sets. These datasets will be linked to the clinical phenotype of an individual. Participants may want to know whether the data obtained on their sample is linked to a better treatment of their condition. Although we do not foresee that this research will deliver immediate new treatments, it will contribute vastly to the ever expanding knowledge on atrial fibrillation.

However, although there will be no direct benefit to the patients, the possible future benefit to the whole cohort should be weighed against the relatively small

risk to the patient as the only invasive procedure in the study is a blood sample, which is taken at the time of routine blood samples needed for the patient's medical care.

**Conflict of Interest**

Participation in this study will be entirely voluntary. There is no vested interest to any of the clinical team in this study that could affect the care and rights of the participants involved.

**Feedback of results**

It is not planned to routinely feedback the results from genetic or other tests obtained from the donated samples. However, a summary of study findings will be sent out to patients who indicate that they would want to receive this at the time of consent.

Furthermore, if the research does identify a cause for atrial fibrillation, stroke or venous thromboembolism, the clinical care team and or/GP would be informed. The participant would be directed to an accredited laboratory for confirmation of the results.

If we discover anything that has an immediate impact on the participant's healthcare, we would ask their clinical care team or GP to invite them for a further sample that will be re- tested in an accredited laboratory (e.g. in case the analysis of their blood sample reveals severe anaemia or abnormal white cell counts). Patients will be asked on the informed consent form for permission for us to contact their GP regarding their participation in the study and the dissemination of any abnormal findings.

## 7 SAFETY CONSIDERTIONS

The main risk is associated with venepuncture. Every venepuncture has a potential for minor problems including bruising, inflammation, and fainting, and taking blood samples may cause discomfort.

However, most patients will be familiar with the process of venepuncture and the person carrying out the procedure would be experienced ensuring that any risk is minimised. Where possible, blood samples will be taken at the same time as blood is collected for the patient's routine care so there is no additional discomfort or risk to the patient. A separate venepuncture for obtaining a sample for the study is not considered to introduce any additional risks to the participant as the blood volume is small and the sample is obtained by an experienced, trained professional.

A small proportion of participants may experience distress/inconvenience when reading the information sheet. However, we ensure that all materials are in plain English and easy to understand and do not obscure the implications of research participation. The information sheet is based on NHS guidance as well as numerous information sheets that have been used for research studies at the

University of Cambridge, Department of Haematology and have been successfully used in the past. The participant information leaflet has also been reviewed by a patient representative.

Participation in the study will not require any changes in the treatment the patient receives. Participation in the study does not require any changes in the medication the patient is taking or is prescribed due to their disease state.

## 8 SAFETY MONITORING, REPORTING AND AUDITING

Access to annonymised data for genotype of phenotype data will only be for genuine researchers through a data access committee.

## 9 FINANCE AND INSURANCE

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