TRINITY HEALTH
KIDNEY CENTRE

Rare Kidney Disease Registry & Biobank
Protocol Version 8

Supported by:

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The Meath Foundation
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Irish Kidney Association – Be a Donor

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I. Part A. Study Introduction

A.1 Abbreviations

RKD	Vasculitis Ireland Network
TCD	Trinity College Dublin
ANCA	Anti neutrophil cytoplasm antibodies
BBV	Blood-borne viruses (such as HIV, Hep B, Hep C)
CKD	Chronic Kidney Disease
CRF	Case Report Form
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid (DNA)
eCRF	Electronic Case Report Form
ESKD	End Stage Kidney Disease
FCS	Fetal Calf Serum
GCP	Good Clinical Practice
GDPR	European General Data Protection Regulation
GMB	Anti-glomerular basement membrane
HBV/HCV	Hepatitis B or C
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
IgA	Immunoglubulin A
PAN	Polyarteritis Nodosa
PBMC	Peripheral Blood Mononuclear cells
PI	Principal Investigator
PIL	Patient Information Leaflet
SOP	Standard Operating Procedure
TCD	Trinity College Dublin
TTMI	Trinity Translational Medicine Institute
HSE	Health Services Executive
CDC	Centre for Disease Prevention and Control

A.2 Investigator Signature Page

I agree to conduct the study according to this protocol (subject to any amendments), and with all locally applicable regulatory requirements, including the Declaration of Helsinki and Good Clinical Practice guidelines.

I agree to conduct the study in person or to supervise the study.

I agree to ensure that all who assist me in the conduct of the study have access to the study protocol plus any amendments and are aware of their obligations

Name of Chief Investigator: Professor Mark Little Title: Consultant Nephrologist

Signature of Chief Investigator: **Date:**

_____ _____

Name of Site Principal Investigator: _____

Title: _____

Signature of Principal Investigator: **Date:**

_____ _____

A.3 Site Responsibilities

Each site is responsible for study implementation and associated patient and study site management. These responsibilities include:

- Patient protection: ensuring the dignity, rights and safety of the patient are upheld and maintained
- Accurate patient identification
- Adherence to eligibility criteria
- Appropriate consent
- Achieving target recruitment
- Staff training and delegation to the study, with maintenance of local delegation log
- Timely and accurate data collection and entry
- Ensuring that all study protocols are adhered to
- Sample collection, transportation, processing and storage at local sites.

A.4 Rare Kidney Disease & Vasculitis Group Statement

All research conducted by the Vasculitis Study Group will adhere to the ethical and scientific quality standards of Good Clinical Practice (GCP). This study manual provides reference and guidance to support the collection of health data and bio-specimens from patients for research.

The following are core guiding principles:

- The safety, rights and wellbeing of all study participants are of primary importance.
- The right to privacy and confidentiality for all study participants will be adhered to in accordance with national data protection guidelines and the GDPR.
- Studies will be conducted in compliance with the study protocol, which will have been authorised by an appropriate ethics committee.
- No clinical study will commence without prior approval from the local research ethics committee.
- The approved study protocol will be adhered to at all times. In the event of a study amendment, it will not be implemented without prior approval from the local research ethics committee.
- All personnel conducting the study will have had an appropriate level of education, training and experience necessary for study procedures.
- All data will be recorded, handled and stored for study purposes in accordance to the principles of GCP and study protocol requirements to ensure accurate reporting, interpretation and verification of data.

A.5 Study Contact Details

Chief Investigator:

Prof Mark Little (Nephrology lead)
Trinity Health Kidney Centre, Tallaght Hospital, Tallaght, Dublin 24
Email: mlittle@tcd.ie

Biobank Coordinator:

Dr. Alan Kennedy (Senior Biobank Technician) Room 1.06, Trinity Translational Medicine Institute, St. James Hospital Dublin 8.
Email: rkdbiobank@tcd.ie

Lead Research nurse:

For any data collection or procedural queries:
Caroline Kosgei
Email: rkdnurse@tcd.ie

Local study site contacts

Tallaght Hospital:

Prof Mark Little (Consultant Nephrologist)
Tallaght Hospital, Dublin 24
Email: mlittle@tcd.ie

Local Research nurse:
Caroline Kosgei,
Email: rkdnurse@tcd.ie

St Vincent's University Hospital:

Dr Eamonn Molloy (Rheumatology Lead)
Dept of Rheumatology, St Vincent's University Hospital, Dublin 4
Email: e.molloy@st-vincents.ie

St. James's Hospital:

Dr Niall Conlon (Consultant Immunologist)
St James' Hospital, Dublin 8
Email: NiaConlon@stjames.ie

Beaumont Hospital:
Prof Mark Little
Beaumont Hospital, Dublin 9
Email: mlittle@tcd.ie
Local Research nurse:
Ms Claire Foley, cfoley@rcsi.ie

Galway University Hospital:
Prof Matt Griffin (Professor of Transplant Immunology)
Galway University Hospital
Email: matthew.griffin@nuigalway.ie

Cork University Hospital:
Dr Mike Clarkson (Consultant Nephrologist)
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Local Research nurse: Aisling Murphy

Limerick University Hospital:
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A.6 Study Overview

A.6.1 Investigation of rare diseases

People with rare diseases (those with a prevalence <5 in 10,000) are served poorly by health providers. As most doctors see few cases of a given rare disease in their entire career, significant delays in diagnosis are usual, with patients receiving treatment for other conditions before a unifying diagnosis is made. Even then, care is fragmented and poorly coordinated: 80% of rare diseases are genetic in origin affecting multiple organ systems, requiring input from multiple specialties on multiple hospital sites, which often lack the specific expertise to deal with these unusual conditions.

Furthermore, research into rare diseases is extremely challenging. Even large centres will see very few patients with a given rare disease, so it is virtually impossible to develop a sufficiently large cohort for meaningful study. Individually, rare diseases do not have a large impact nationally, so they are ignored by funding agencies, which favour supporting heart disease and cancer research. However, 1 in 17 people will be affected by a rare disease at some point in their life, equating to 250,000 people in Ireland. Therefore, collectively they represent a very significant burden to health services.

To share expertise and resources, networked collaboration between units is essential. For a country the size of Ireland, a national network would be of the right size and would allow study of epidemiology across the whole country. Such a network would revolve around a robust patient registry, with capture of detailed longitudinal clinical data across multiple units and linked collection of biological samples to facilitate in depth study of a large cohort from all parts of Ireland.

A.6.2 Systemic Vasculitis: networked investigation of a rare disease

This affects about 20 per million population per year, as opposed to affecting a handful of people in the country, so is considered a “common-rare disease”. It causes severe multi-organ dysfunction, including irreversible kidney failure, lung haemorrhage, stroke and sino-nasal destruction. The prevalence is 300/million and hence can only be studied by a coordinated international network of centres. The Vasculitis Ireland Network (formerly ‘The Renal Kidney Disease, RKD, Bioresource’) was created in 2014 to harmonise care for vasculitis patients across Ireland. It is a member of ERN-RITA, the European Reference Network for rare immune disorders. Documenting and reporting on clinical outcomes and benchmarking against international norms using a dedicated registry are key components of ERN membership.

The primary aims of this initiative are to address fundamental questions about vasculitis epidemiology, facilitate conduct of phase II/III interventional studies by allowing easy identification of a suitable cohort, monitor use of novel biologic agents, compile sufficiently large cohorts to study immunogenetics, rapidly assess the clinical utility of new biomarkers for development in clinical trials as surrogate end-points, and to characterise ‘difficult to

define' disease subgroups including ANCA negative vasculitis and polyarteritis overlap syndromes.

A.6.3 Study Design

The primary objective of this study is to understand more about systemic vasculitis by forming a national network of interested units.

The aims of the study are to:

1. Establish and maintain a registry for vasculitis.
2. Use the registry to study clinical outcomes for systemic vasculitis.
3. Collect and store biological samples (serum, urine, leucocytes and DNA samples) and use these in concert with the registry data to identify susceptibility genes and proteins for the disease.
4. Share Coded samples and data with external researchers for research into vasculitis.

A.6.4 Endpoints

This is a sample ('biobank') and data ('registry') repository for ongoing research in the area of vasculitis. The intention is to understand more about causes and progression with the long-term goal of research into better diagnostic tests/treatments. In common with other biobank initiatives, the study endpoints cannot be clearly defined as the research will evolve over time and the experimental questions cannot be predicted at this time.

The endpoints will include:

- Patient and renal survival,
- Time to End Stage Kidney Disease,
- Time to relapse following treatment,
- Occurrence of severe complications of the disease or its therapy.

A.6.5 Study Participants

Patient Group:

The aim is to invite >90% of incident patients around Ireland to participate (about 90 incident patients per year). There are approximately 1000 patients in Ireland living with systemic vasculitis; we aim to recruit all of these cases. Participant recruitment is described in detail in section B1.1 & B2.1 (Recruitment at sites with and without designated Research nurse).

Control Group:

1. Unaffected relatives: Participants will be screened for the disease as it is possible that they may have subclinical manifestations, or they could theoretically develop the disease at some stage in the future. Screening will involve urinalysis: a negative result for blood, nitrite and protein will allow for recruitment onto the study when combined with self-reported absence of known history of kidney disease.

2. Healthy controls (age matched related and unrelated spousal): Participants will be screened for disease by asking about previous medical problems and performing urine dipstick. Healthy controls should be on no long-term medication. Vasculitis has a roughly 50:50 gender split so we aim to replicate this in the control groups.

3. Disease controls: In recruiting this group we aim to include participants with renal impairment and/or non-vasculitis autoimmune disease (requiring immunosuppressive medication) and/or systemic infection. Where possible, these should be age matched and approximately 50:50 gender split.

A.6.6 Study Methodology - Overview

Patients with vasculitis will be identified, where possible, at the time of diagnosis and before induction treatment has been initiated. This entails requirement for a nimble and responsive recruitment process to allow recruitment at short notice and outside office hours. Prevalent patients will also be recruited from site clinics during attendance for routine clinical care. In brief:

1. Identification of a patient with a new diagnosis of systemic vasculitis at one of the participating sites is communicated to the lead Research nurse and biobank team.
2. The local recruiting team is supported in seeking consent from the patient.
3. Once samples have been obtained, a courier transports them to the TCD biobank, where they are processed and stored.
4. In the week following recruitment, the lead research nurse enters clinical data into the RKD registry database and validates appropriate completion of the local recruitment log and storage of consent forms.

In addition, dedicated research clinics will operate at several sites, including Tallaght, St James's, Beaumont and Cork hospitals. The main function of these is to obtain follow-up samples and data from recruited patients, and to identify and recruit the occasional patient with prevalent vasculitis who has not yet been recruited.

Patients will also be asked to invite unaffected related and spousal controls to participate. These control patients will be screened for disease and consented in the normal fashion. Their participation is vital to the success of identifying disease causing genes as examining their DNA can help differentiate disease causing gene changes from harmless familial changes. In addition, they will act as controls in research investigating the pathogenesis of vasculitis, and in biomarker studies.

A.7 Ethical Considerations

It is possible that a genetic predisposition to develop disease will be discovered during this research. In patients who have already presented with the disease and are engaging in clinical care there is little ethical issue. However, it may be possible that a family member who was

previously considered unaffected carries a disease gene. Unaffected individuals will be informed of this risk on an information sheet and during the consent process. This raises several ethical issues:

- It is possible to carry a disease phenotype and not manifest the disease. This depends on the penetrance of the phenotype.
- It is also possible that there may be no immediate therapy available for any disease gene identified.

In these situations, it is imperative to balance the benefit of telling the individual their genotype with the potential anxiety caused and whether they wish to know and the potential for intervention.

In such a case,

- A generic letter would be written to all the family members with information specific to the gene identified in non-medical language.
- All individuals would be invited to make an appointment to discuss their results. Individuals who would wish to discuss their results would have genetic counselling prior to this and they would then have the genetic tests confirmed in a clinical genetics setting.
- They would then be discussed at an appropriate level of detail by the PIs and an appropriate medical screening plan along with any appropriate investigation or care plan would be made at that stage.

A.8 Governance and Quality Oversight

A.8.1 RKD Registry Steering Group

The RKD Registry and Biobank is managed by a steering group comprising a representative from those units that contribute >10% of cases plus several lay members and is chaired by a respected independent researcher with experience of this work. This group works to maintain and build this resource for the public good in accordance with its purpose and in accordance with regulatory requirements.

The Committee retains full control of all access to and uses of this resource. Any application for access will be reviewed to ensure that it is consistent with the participants' consent, that they have the relevant ethics approval and that there is a sound scientific basis.

A.8.2 RKD Registry Steering Group Members:

The steering committee is currently comprised of:

- Joe McPartlin (Independent Chair)

- Mark Little (Chief Investigator)
- Alan Kennedy (Biobank manager)
- Julie Power (Patient representative)
- Peter Conlon (Chief Investigator of Irish Kidney Gene Project)
- Jane Richardson (Lead Research nurse)
- Eamon Molloy (Consultant Rheumatologist)
- Matthew Griffin (Consultant Nephrologist)
- Michael Clarkson (Consultant Nephrologist)
- Tony White (Patient representative)
- Fionnuala Hickey (Independent senior researcher)

Changes to membership will be approved by the Steering Committee.

A.8.3 Study Monitoring/Quality Review

Study files (Recruitment log and eCRF) will be reviewed periodically by the PI or nominated reviewer. All data points should be completed. The Lead Research nurse will act as liaison between the various hospital sites and the PI to ensure that:

- Study is being conducted according to protocol and any applicable amendments
- Data are collected and recorded accurately
- Informed consent is obtained before any study related procedures are carried out
- Quality and completeness of informed consent documentation and recruitment logs
- Study patients meet with the eligibility criteria
- Study site file is up to date.

Further follow-up with sites may be necessary if persistent protocol deviations are observed, such as re-training or additional guidance.

A.9 Eligibility Criteria

A.9.1 Patient Group – Inclusion and Exclusion Criteria

Inclusion Criteria

1. Diagnosis of one of the following forms of systemic vasculitis:
   - (i) Small vessel vasculitis (ANCA associated):
     - a. Microscopic polyangiitis (including renal limited vasculitis)
     - b. Granulomatosis with polyangiitis (Wegener)
     - c. Eosinophilic granulomatosis with polyangiitis (Churg Strauss)
     - d. ANCA vasculitis unclassified
   - (ii) Small vessel vasculitis (Immune complex)
     - a. anti-GBM disease
     - b. Cryoglobulinemic vasculitis
     - c. IgA vasculitis (Henoch-Schonlein)
   - (iii) Medium vessel vasculitis:
     - a. Classical PAN
     - b. Kawasaki disease
   - (iv) Large vessel vasculitis:
     - a. Giant cell arteritis
     - b. Takayasu's arteritis
   - (v) Variable vessel vasculitis:
     - a. Behcet's disease
     - b. Cogan's syndrome
   - (vi) Single organ vasculitis:
     - a. Isolated aortitis
     - b. Primary cerebral angiitis
2. Capable of understanding and complying with the requirements of the protocol, including ability to attend study clinic AND willing to provide signed and dated written, voluntary informed consent before any protocol specific procedures are performed.
3. Age 16 years or over

Exclusion Criteria:

1. Not willing to attend one of the research clinics
2. Age under 16 years

A.9.2 Control Group – Inclusion and Exclusion Criteria

Inclusion Criteria

1. Unaffected family members.

2. Spouse/partner living in same environment.
3. Unrelated age-matched healthy control - an individual who does not have vasculitis and does not share the same environment. Ideally, to match the Vasculitis Patient Cohort, the Healthy controls should be above 50 years old.
4. Disease Control Individual- diagnosis of a disease other than Vasculitis and in most cases also on immunosuppressive medication.
5. Capable of understanding and complying with the requirements of the protocol.
6. Capable of understanding and willing to provide signed and dated written, voluntary informed consent before any protocol specific procedures are performed.

Exclusion Criteria:

1. Not willing to attend one of the research clinics
2. Age under 16 years

II. Part B. Study Procedures

B.1 Informed Consent

B.1.1 Informed Consent Principles

In line with the Declaration of Helsinki and ICH Good Clinical Practice guidelines:

- The rights, safety and wellbeing of the subjects are the most important consideration and should prevail over interests of science and society.
- Freely given informed consent should be obtained from every subject prior to participation.

Note: This study is NOT a clinical trial

Informed consent will be sought from all participants and will be obtained by one of the lead investigators or a fully qualified, trained **AND** delegated member of the research team such as a Research nurse or research registrar.

Consent will encompass the nature of the study, consent to review and collate the information from medical records, phlebotomy, and urine sample. They will be informed that DNA and proteins will be extracted from blood, plasma and urine samples and that a small portion of the biopsy sample obtained for clinical purposes will be used for experimental studies. They will explain to the patient that a portion of the urine sample they have provided for clinic will be used for the study.

Risks and benefits of participation will be discussed, and patients will be made aware that the study is voluntary, and their decision will not alter their healthcare in any way. They will also be informed that they will not have access to the results of analysis of their individual specimens and will be reassured regarding confidentiality.

The patient will decide for themselves whether they wish to participate in the study or not. Their decision-making process will be free from coercion or undue pressure and the patient will be aware that they are free to withdraw from the research process at any time.

As required under the Data Protection Act, explicit consent for use of data will be obtained. Participants need to be informed of their rights under GDPR and advised on withdrawal procedures.

Once satisfied that the patient or their legal representative fully understands the nature and objectives of the study as outlined in the PIL/ICF and is capable of giving informed consent, the patient can be consented and enrolled in the study.

B.1.2 Informed Consent Procedure:

- The correct PIL/ICF must be used:
  - ***Information Leaflet and Consent Form for Patients***
- **Inpatients** will be given a Participant Information Leaflet (PIL) along with an explanation of the study. They will be given enough time to reflect and will have the opportunity to ask questions or speak to relative/GP/friends before being asked to sign the consent forms.
- In the **outpatient** clinic setting, a PIL will be included in the appointment letter. Copies will also be available at the relevant clinic. Patients will be given enough time to consider participating in the study and to ask questions prior to appointment. They can choose to consent at that appointment or can defer to a subsequent visit if they need more time.

B.1.3 Informed Consent Procedure: Healthy and Disease Controls

- The correct PIL/ICF must be used:
  - ***Information Leaflet and Consent Form: Control Subject***
- A PIL (for 'control') may be provided to patients to give to unaffected relatives or spouses, inviting them to contact the research team. Alternatively, unrelated healthy controls will be given the PIL (for 'control') to peruse at their leisure.
- If interested in participating, controls will be invited to attend the research centre.
- The study will be explained to potential participants and they will be given the opportunity to ask questions.
- Informed consent will be obtained before any study-related procedure is carried out.

B.1.4 Informed Consent Procedure: Prevalent Patients under 18 years

- This study will include participants aged 16-18 years. Parental consent will be required for participants under 18 years.
- The correct PIL/ICF must be used:
  - ***Information Leaflet and Consent Form: Parental Consent***
- Consent of a parent or legal guardian must be obtained
- The study will be explained to the participant and their parent/ legal guardian and both will be given the opportunity to ask questions
- The parent or guardian cannot provide consent unless the participant assents to being included.
- Once the participant turns 18, they will need to be re-consented as per B.4.1.

B.1.5 Documenting Consent

B.1.5 (a) Documenting Consent on the Informed Consent Form

1. Patient information leaflets are available to download at: https://www.tcd.ie/medicine/thkc/research/rare.php
2. The Informed Consent Form is included in the final 2 pages of the PIL
3. The correct PIL/ICF must be used. There are three different forms:
    a. PIL/ICF for patients (i.e. participants with vasculitis)
    b. PIL/ICF for patients requiring parental consent (i.e. 16-18 year olds)
    c. PIL/ICF for controls (i.e. participants without vasculitis)
4. Consent can only be obtained if the participant agrees with all statements on the ICF.
5. Agreement to all statements must be indicated by initialling each box.
6. A site code and unique study number will be issued and written on the consent form.
7. The participant/ parent must personally print name, sign and date their section of the ICF.
8. The person obtaining consent (Research nurse/ study doctor) must personally print name, sign and date the ICF.
9. Two (2) copies of the original PIL/ICF must be made (photocopy) – original is filed in the site file/ study folder, one copy is filed in the medical record and one copy is provided to the participant/ legal representative/ parent.

B.1.5 (b) Documenting Consent in Patient Medical Records

When a patient is approached to participate in this study and gives their consent, information pertaining to the study enrolment should be documented in their medical notes:

1. Clearly state the name of the study "RKD Registry and Biobank".
2. State the date that the PIL/ICF was provided to the participant.
3. Clearly state that the patient gave informed consent and include a copy of the ICF.
4. Clearly state the date the consent was given and the version of PIL/ICF.
5. Clearly indicate what samples were collected. If there were any issues with samples being collected, please detail these.
6. In case of assent from a legal representative or parent, clearly document the name of the person assenting, relationship to the patient, any discussion about the study, version of PIL/ICF, date and time of the consent.

B.2 Research Nurse Designated Sites

B.2.1 Recruitment at sites with a designated Research nurse

1. Participants can be recruited as Patients or Controls.
2. Prior to the collection of clinical data or any study samples being obtained informed consent is obtained (Figure 1).

3. The method in which the Research nurse interacts with the participant will vary at each site, depending on how the clinic operates locally. A suggested method is for the Research nurse to approach the patient as they arrive at clinic. The patient should have received a patient information leaflet in the post prior to this. The Research nurse will ask the patient if they have had time to consider participating.
4. If they are willing to provide informed consent the Research nurse will escort the patient to a private area to explain the study in more detail and provide an opportunity for any questions to be asked.
5. Informed consent obtained AND documented as per Section B.1.
6. RKD study ID is assigned by PI or delegate. These are determined by the next available record ID in the RKD REDCap database.
7. After obtaining informed consent the research nurse will collect the demographic data for the eCRF.
8. Collect samples as per section B.6 (Sample collection)
9. Complete recruitment log (see B.4).

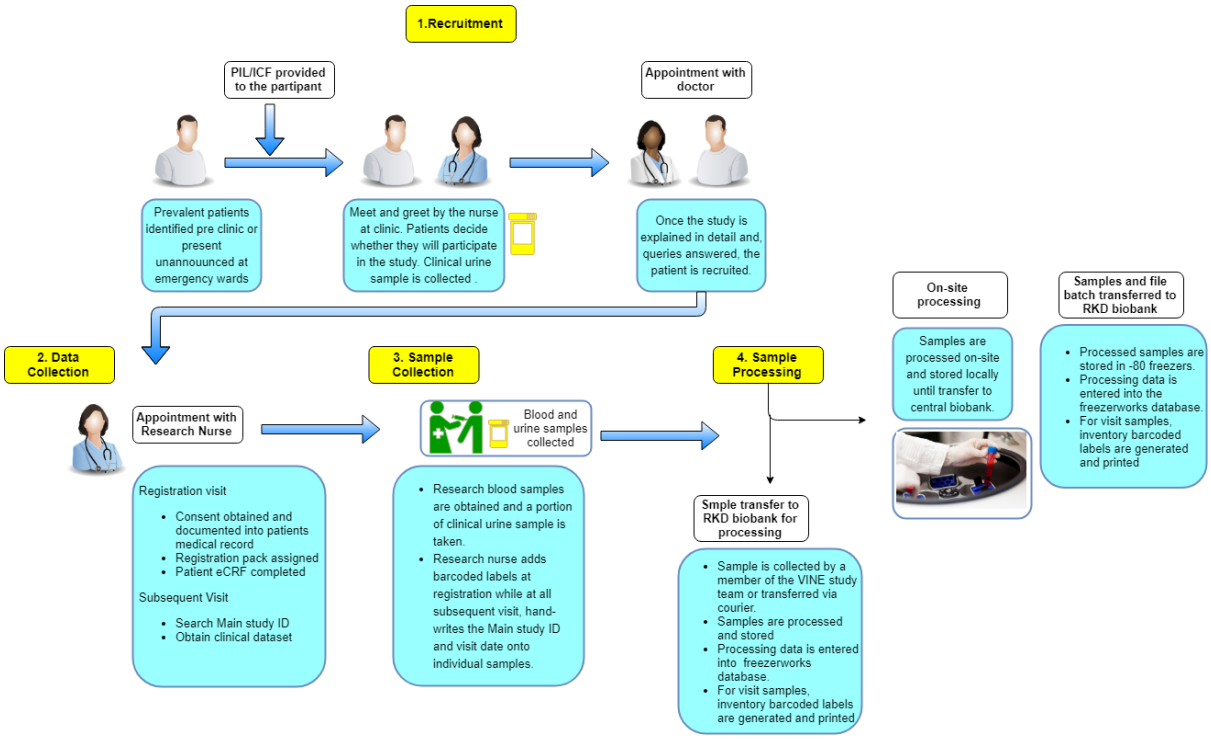

Figure 1. Summary schema of Recruitment and Sample management at sites with a designated research nurse.

B.3 Sites without designated Research Nurse

B.3.1 Patient Recruitment at sites without a designated Research nurse.

1. Upon making a clinical diagnosis of acute vasculitis, inform RKD study team of the potential recruit by phoning, texting or emailing Prof. Little, (mlittle@tcd.ie or tel: 086-6096068) or by placing a message on the "Acute Vasculitis" WhatsApp group. **Do not include any patient identifiable information in the message.**
2. Information leaflets and consent forms can by obtained here: https://www.tcd.ie/medicine/thkc/research/rare.php
3. A member of the local recruitment staff will approach the patient and provide them with the patient information leaflet and give them adequate time to consider participation (Figure 2).
4. If they are willing to provide informed consent the participant should be brought to a private area to explain the study in more detail and provide an opportunity for any questions to be asked.
5. Obtain AND document informed consent as per B.1.
6. A study ID will be provided by the RKD study team once informed consent is obtained.
7. Following informed consent, collect the demographic data for the eCRF.
8. Explain that a portion of the urine sample provided for clinical use will be used for the study.
9. Explain that blood samples will also be collected for the study, preferably before immunosuppression treatment is commenced, as per section B.6 (Sample collection).
10. Complete recruitment log (see B.4).
11. The lead research nurse will contact or visit the site 7-10 days later to input outcome data into the eCRF. This should be collated after reference to the medical notes and discussion with the medical team caring for the patient.

B.3.2 Sample transfer and processing

1. These sites do not have a clinical research facility to process the samples, so the samples are transported to central RKD biobank at TTMI.
2. Samples will be collected by a courier within 4-12 hours of sampling (if overnight) and transferred at 4°C to central RKD biobank site for processing. The courier will be organised by the biobank technician or a member of the RKD group. Samples should be stored at 4°C (ie. In a refrigerator) until collection.
3. Immediately upon arrival onsite, the biobank technician/local member of the RKD group will unpack the samples and ensure all samples have main study ID clearly written on them.
4. Samples will be processed according to the SOPs described in the sample processing lab manual. Briefly, plasma, serum and urine samples will be processed and aliquoted into vials assigned with barcoded labels for the corresponding main study ID.

5. Aliquoted samples will be stored in RKD biobank's -80°C freezers. Biobank technician will then enter the aliquot details in a simple inventory system mapping the location of the samples in the freezers.

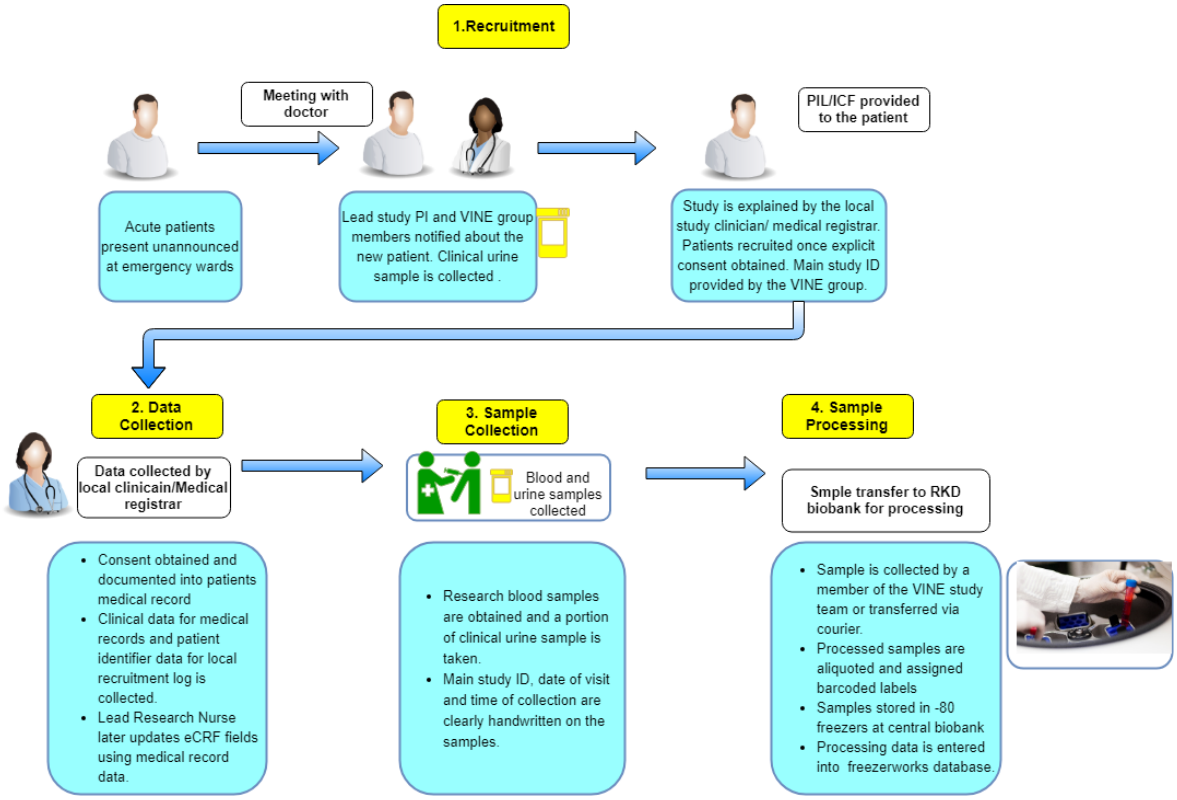

Figure 2. Summary schema of Recruitment and Sample management at sites without a designated Research nurse

B.4 Patient Recruitment Log - Completion Guidelines

This document contains patient identifiable information and is a link between an individual and their unique anonymised study number. The log should be held in electronic format securely (on an access controlled shared folder or in a password protected file on a desktop computer) at the host site. These data are essential so that the patient can be followed-up and longitudinal clinical information linked to subsequent samples.

The following information should be collected:

- Full name (First name, surname)
- Date of birth
- Hospital MRN
- Contact phone number
- Email address

- Site code
- Study specific number
- Full name of GP
- Address of GP
- Phone number of GP

A central non-identifiable national record will also be maintained by the lead research nurse to ensure complete data capture. This will track when data is obtained, including: initial phenotyping, biopsy report and any missing data which requires follow-up at the next site visit.

Confidentiality

A unique patient identifier (study ID) should be recorded on the patient's consent form, recruitment log, eCRF and any study related documents. This will consist of a unique study specific number, which will be pre-generated and will serve as the unique identifier for both patient samples and clinical data held outside the study site. Data are thus pseudonymised, with re-identification possible via a recruitment log.

B.5 Data Collection & Site File Management

A study site file will be prepared containing all critical documents pertinent to the study, including:

- Protocol
- Ethics approval letters
- Patient recruitment Logs
- PIL/ICF documents

A local recruitment log with identifiable patient data will be maintained at each local and peripheral site (see template). The recruiting doctor/Research nurse/research registrar will record the patient's details (name, date of birth, address, contact number and corresponding study ID) on the log at the time of recruitment. Ideally this will be an excel file, maintained behind a secure firewall.

B.6 Sample Collection

The Research nurse/local recruitment staff will have completed the appropriate training and be deemed competent in the procedure of venepuncture prior to any blood collection in accordance with local policy and procedures and will also be competent to respond in the event of fainting or any other adverse event during or after the blood collection procedure.

Summary of order of sample collection (cap colours may vary from site to site)

Blood:

1 Serum tube (red cap); room temp

2 EDTA tubes (purple cap); on ice or in refrigerator

2 Li Hep tubes (if requested, green cap); room temp

1 PaxGene tube (clear cap with red rubber on inside), leave at room temp, keep vertical

Urine:

1 Sterilin – pour 10mL into 2 x 50mL tubes and place on ice or into refrigerator

Transport to lab for processing as quickly as possible. Fill in sample processing sheet.

Blood tube descriptions

EDTA Plasma purple top tube (ethylene diamine tetra acetic acid).
A colourless crystalline acid that acts as a strong chelating agent, forms a sodium salt used to keep blood samples from clotting before tests are run: an anticoagulant.
Can be used to collect blood for DNA extraction, PBMC preparation and Plasma.

Serum Collection Tube Red top: contains clotting activators to encourage the clotting process to enable collection of serum 30 minutes after collection.

PAXgene Blood RNA (clear top with rubber/brown insert)
The PAXgene RNA tube will be used for blood collection, RNA stabilization, specimen transport and storage. It is prefilled with an RNA stabilization reagent to provide immediate RNA stabilization. The blood cell lysis in the tube simplifies subsequent RNA purification. It also allows for consistent blood draw volume and blood-to-additive ratio.

B.6.1 Procedure for Urine Sample Collection

1. As part of normal clinical care, the patient will be asked to provide a urine sample. (The Research nurse/recruitment staff can offer a glass of water at the patient's first arrival to the clinic to improve the ability of the patient to provide this volume of urine). 20-30mL of this urine sample is reserved for research. The urine sample should be placed on ice.
2. Label the sides of the urine tubes with RKD ID and date
3. Add 10 ml of the urine into a red capped 50ml tube. Label this tube with the Main Study ID. Write 'Urine-M' on the sides and the lid of the tube. Place this tube immediately on ice.
4. Record the time of collection in the sample details section of the sample processing sheet provided in the pack OR clearly hand write on the tube labels.

B.6.2 Procedure for Blood Sample Collection

1. Position the patient safely and comfortably in a chair/couch, ensuring that the arm is in the correct position to draw blood.
2. Ensure that the patient's mouth is free from any food or gum.
3. All sample containers and equipment should be assembled prior to the procedure.
4. Research nurse/recruitment staff should always wear gloves and appropriate barrier protection during the venepuncture and follow the standard local safety guidelines for blood collection.

   A video instruction of the safety precautions if collecting a PaxGene tube can be viewed at: http://www.preanalytix.com/videos/rna-tube-collection-video/

5. **Order of Draw:**
    a. Collect 9ml of blood directly into a vacutainer blood collection tube containing **serum clot activator (red top),**
    b. Two 9ml tubes of blood into a vacutainer blood container containing **EDTA (purple top) for plasma and DNA**,
    c. Two 9mL tubes of blood into a vacutainer blood container containing **Li Hep** (if required).
    d. Finally 2.5ml should be collected into the **PAX gene RNA tube** (if required).
6. Allow **at least 10 seconds for a complete blood draw** to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder**.**
7. Filling the blood collection tube to the black mark on the tube label indicates that the correct amount of blood has been drawn. **Under-filling or over-filling of the tube may affect laboratory results due to the incorrect blood/additive ratio.**
8. Immediately after blood collection, gently invert all the blood collection tubes 10 **times** to mix the additives with the blood and to prevent formation of fibrin which may affect subsequent analysis.
9. Write the time of collection on each tube.
10. Handwrite the Main study ID on each tube.
11. **DO NOT PLACE THE SERUM (red top) or PaxGene tube ON ICE.** Place the Serum (red top) tube at room temperature for 30 minutes to clot. Store the PaxGene Blood RNA Tube **upright** at room temperature while transporting to the lab.
12. Place the EDTA tubes (for plasma and DNA) immediately into a polystyrene/'cooler' box filled with ice/ice packs to maintain it at 4°C until processing and storage at -80°C.
13. Record the time of collection in the sample details section of the sample processing sheet provided in the pack OR clearly hand write on the tube labels.

Note: *Venous blood samples may be obtained via direct venepuncture or via other available venous access (e.g., an existing peripheral intravenous line or hep-lock) – if the hospital staff follows their protocol for first withdrawing blood to flush the line.*

B.6.3 Procedure for PaxGene RNA Collection

1. If the PAX gene Blood RNA Tube is the only tube to be drawn, a small amount of blood should be drawn into a "Discard Tube" prior to drawing blood into the PaxGene Blood RNA Tube. Otherwise, the PaxGene Blood RNA Tube should be the last tube drawn in the phlebotomy procedure.
2. Hold the PaxGene Blood RNA Tube vertically, below the blood donor's arm, during blood collection and make sure tube additives do not touch stopper or end of the needle during venepuncture.

B.6.4 Healthy Control Registration Encounters:

All Healthy controls should have a urine sample tested, ideally with the following dipsticks: Siemens Multistix 10 SG REF 2300 (0353597), illustrated below. It is important that the dipstick used is capable of recording nitrite, blood and protein to ensure that there is no urinary tract infection.

B.6.5 SAMPLE PROCESSING SHEET

The Research Nurse will write the **Main Study ID Number** here **and on every page** of the Case Report Form.

The Research Nurse will indicate the **encounter number** here

The Research Nurse will indicate the **type of patient** here

The Research Nurse will write the **Date of Visit** here on page 2 of the Case Report Forms

Main Study ID..... UKIVAS ID.....
SITE..... Patient Initials.....

Section 3	SAMPLE DETAILS		
Encounter (please tick)	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	other:_____	
Type of Participant	Patient ☐	Disease Control ☐	Healthy Control ☐
Date (dd/mmm/yyyy)	__/___/____		
UKIVAS ID		PAXgene tube	Time of Collection:
Main Study I.D.			Time of Freezing:
Collected by:		BDP100 Proteomics	Time of Collection:
Processed by:			Time of Freezing:
Date:			
URINE DETAILS	TIME OF VOIDING:	IS THIS URINE FROM A CATHETHER BAG	YES NO (please circle)
TRANSPORT TEM	ICE /4	ICE /4	ROOM TEMPERATURE
Urine Sample type:	Proteomics	Metabolomics	Exosome
TIME OF PROCESSING			
TIME OF FREEZING			
Total Volume (ml):			
No. of 5ml (50ml for Exosome) tubes frozen:			
Volume of each 5ml (or 50ml) tube:			
Number of 2ml cryovials frozen:			
Volume in each cryovial:			
Protease Inhibitor tablet added	Yes/ NO- Please Circle		

SERUM DETAILS		PLASMA DETAILS	
TIME OF COLLECTION:		TIME OF EDTA TUBE COLLECTION:	
TIME OF SPINNING:		TIME OF SPINNING:	
TIME OF FREEZING:		TIME OF FREEZING:	

B.6.6 Sample transfer and processing

On-site Processing

- Samples will be processed according to SOP described in the lab manual provided at these sites.
- Processed samples will be frozen at -80 °C (or initially -20 for PaxGene tube) at the local site. This will usually involve placing all the samples from one patient encounter to be stored at -80°C together in a purposely supplied Ziploc Bag, writing the main study ID and the visit date on the front of the bag and placing it in a box marked RKD samples in the -80 °C Freezer.
- Once enough samples to warrant a collection visit are accumulated at a local site, transfer to RKD biobank at Trinity Translational Medicine Institute, St. James' Hospital, while maintaining correct transport and storage temperatures will be arranged (using dry ice).
- The processing sheets will be retained securely by the Research nurse/biobank technician, transported, and filed at the central biobank.

Off-site processing

- In certain cases of **Acute patient recruitment**, the samples are transferred to RKD biobank directly for processing.
- In most cases, samples will be collected by a member of the RKD study team (to reduce arm to processing time for these samples) OR collected by a courier within 4-12 hours of sampling (if overnight) and transferred at 4°C to central RKD biobank site for processing. The courier will be organised by the biobank technician or a member of the RKD group.
- Samples will be processed according to the SOPs described in the sample processing lab manual. Briefly, plasma, serum and urine samples will be processed and aliquoted into vials assigned with barcoded labels for the corresponding Main study ID.
- Aliquoted samples will be stored in -80°C freezers. Biobank technician will then enter the aliquot details in a simple inventory system mapping the location of the samples in the freezers.

III Part C. Data management

C.1 Data Management

C.1.1 eCRF Completion Guidelines

- All information should be recorded, handled and stored in a way that allows accurate reporting, interpretation and verification.
- The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality.
- Data recorded in the eCRF, which are derived from source documents, should be consistent with the source documents.
- Anonymised clinical registry data should be recorded electronically in the eCRF (REDCap).

C.1.2 Data Security

- Identifiable patient/control data is pseudonymised after recruitment by assigning a study ID; their consent forms with medical record numbers will be stored in a secure facility at the local hospital site.
- Pseudonymised data will be uploaded on the eCRF database which will be mapped to a dedicated password protected computer using IP address. The database will be protected behind host and institutional firewall with access to dedicated personnel only.
- Coded biological samples are processed and stored centrally at the RKD biobank and archived by the biobank technician using industry standard Freezerworks software; only the biobank technician and Lead study PI have access to the software.
- For further information please refer to Data Protection Impact Assessment.

C.1.3 Access to database

- The database operates on multiple-access levels, only the lead study PI and research nurse will have complete access to the database and permission to modify, while other members will have limited access with the rights to upload and read only; new access requests will be approved by the lead study PI.
- The registry database will be shared with college IT service providers as its security and access permissions are managed by them. This database will be periodically backed up on an external third-party server located in Dublin.

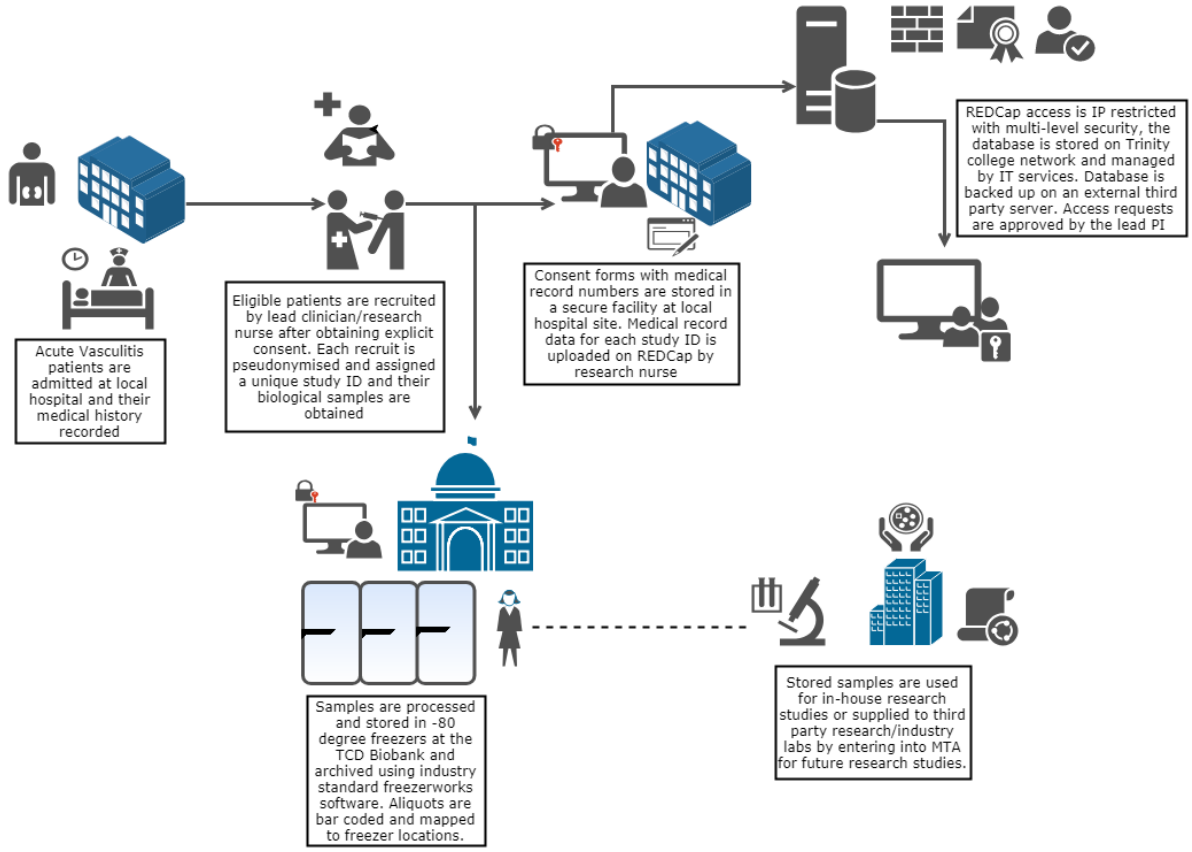

Figure 7. Flow diagram of data security and access.

C.1.4 Withdrawal procedure

- If the patient decides to withdraw from the study at any stage, the Research nurse/research team member will document this decision clearly in the patient's medical notes and CRF and eCRF detailing the reason if known.
- A withdrawal form must be completed.
- Participants have the following options:
  - **No further access:** This means that no further data or samples will be collected. The participant will no longer be contacted. However, the Registry and Biobank will still have permission to use, store and share information and samples collected up until this date.
  - **No further use:** This means that no further data or samples will be collected. The participant will no longer be contacted. Samples held by the RKD Biobank and Registry will be destroyed. Data held on the RKD Biobank and Registry holds on you will be deleted. Researchers show have received samples and data will be contacted to request that unused samples and data be destroyed. Research results from data that has been analysed will continue to be used.

- **No Further Access:**

  - Recruitment log is retained, but an entry is made to no longer follow up on the participant
  - Recorded on study database.
  - ICF and Withdrawal form are retained at site.

- **No further use:**
  - ICF and withdrawal form are retained at site.
  - Recruitment log is updated to indicate that the participant has withdrawn.
  - Samples are recalled from the biobank and destroyed.
  - Study database is updated.

Appendix I: Description of clinico-pathological ANCA vasculitis syndromes

- **Granulomatosis with polyangiitis (GPA)**
  GPA is classically associated with granulomatous inflammation of the upper respiratory tract, which produces a variety of ENT symptoms including nasal crusting, hearing loss (conductive and sensorineural), sinusitis and occasionally a saddle-shaped nasal deformity as a result of necrotising inflammation in the cartilaginous nasal septum. Seventy-seven percent of patients with GPA have upper respiratory tract symptoms at the time of diagnosis compared to 29% of patients with MPA. Ultimately, 86% of patients with GPA will develop renal disease at some point in their disease although this is less common at presentation than in MPA (see below).
  E.g. A patient with pulmonary involvement (e.g. lung haemorrhage or infiltrates on chest x-ray) or granulomatous inflammation on biopsy, and renal involvement (usually c-ANCA and PR3 positive).

- **Microscopic polyangiitis (MPA)**
  If there is no clinical evidence of granulomatous inflammation or eosinophilic vasculitis, the ANCA vasculitis syndrome is termed **MPA**. It is important to note that granulomatous inflammation may emerge at a later time point, at which stage the diagnosis will change to GPA. Extra-renal organ involvement in MPA is generally less common than in GPA. Patients tend to present with renal disease, which is more common at the time of diagnosis in MPA than in GPA (92% vs. 77%), possibly as they present later in the disease because the renal disease is largely asymptomatic until advanced.
  E.g. A patient with no pulmonary involvement and normal eosinophils, with predominantly renal involvement (usually p-ANCA and MPO positive).

- **Single-organ AAV (e.g. renal-limited AAV)**
  Some patients may present with disease limited to the kidney (causing a pauci-immune crescentic glomerulonephritis). This renal limited disease is considered MPA, unless evidence of granulomatous inflammation develops subsequently.

- **Eosinophilic granulomatosis with polyangiitis (EGPA)**
  EGPA displays clinical features of (1) late onset asthma and wheeze (>95%) with (2) variable peripheral blood eosinophilia accounting for >10% of leukocytes (100%, although disappears rapidly with treatment of asthma with glucocorticoids so may be missed) and evidence of (3) end organ damage secondary to vasculitis. Rhinitis, with nasal polyposis and hearing loss is often present. End organ disease is manifested as skin granulomas or palpable purpura (60%), mononeuritis multiplex (75%), pauci-immune crescentic glomerulonephritis (25%) and cardiac disease (pericarditis /

myocarditis / valvular lesions / coronary arteritis, 40%). It is not uncommon for patients to have chest symptoms with eosinophilia for months or years before developing overt vasculitis. Renal involvement is less common compared to GPA and MPA and the association with ANCA is much weaker, although those with renal disease tend to have positive ANCA serology.

E.g. A patient with asthma and high eosinophils on white cell count at the time of presentation, with usually multiple systems involved (nerves, heart, skin, kidney, lungs). These patients more commonly display p-ANCA and MPO positivity.

Table 1: Characteristics of ANCA vasculitis clinico-pathological syndromes

Characteristic	GPA	MPA	EGPA
Granulomatosis	+	-	+
Eosinophilia	-	-	+
Asthma	-	-	+
Upper respiratory tract (URT)/ENT symptoms	+ (77%)	+ (29%)	+ (nasal polyps common)
Lower respiratory tract (LRT) symptoms (e.g. pulmonary haemorrhage)	+ (85%)	+	+

Renal Involvement	77%	92%	Less common (25%, bad prognostic sign)
Other common features			Skin (60%), mononeuritis multiplex (75%), cardiac involvement (40%), GI
Limited subtypes	URT/LRT/eye	Renal limited	URT/LRT
ANCA subtype	PR3 (-ve in 40% of limited GPA)	MPO (70%), PR3 can occur	MPO (30-38%)(8), increased ANCA positivity with presence of renal disease
Incidence	Increased in UK vs China/Japan (14.3 vs 2.1/million adults)	Increased in China/Japan vs UK (18.2 vs 6.5/million adults)	2.4 million/year (least common)

- **Flare events:**
  A possible flare event is characterised by the onset of new or worsening symptoms and/or signs of organ dysfunction (e.g. rising creatinine, new haematuria, worsening proteinuria, weight loss) +/ confirmation of acute inflammation on biopsy. Usually, immunosuppression is increased (induction treatment is repeated in severe cases), with resultant improvement in the patient's condition.

  Flares will be characterised as minor (Birmingham Vasculitis Activity Scale [BVAS] >1) or major (with major BVAS item):
  - **Definite:** Active vasculitis with histopathological confirmation, treated with immunosuppression escalation, and clinical response to same.

- **High probability:** Active vasculitis supported by suggestive clinical features, biochemistry (c-reactive protein, creatinine, proteinuria, ANCA serology), urinalysis (red cells and casts) and/or radiological investigation, treated with immunosuppression escalation, and clinical response to same, but without histopathological confirmation.
- **Possible:** Active vasculitis on physician assessment, but limited supporting laboratory/radiological evidence, treated with immunosuppression escalation, and clinical response to same

Appendix II: Explanation of typical treatment regime

- The typical treatment regime is described below.
- The researcher may need to request medical records (i.e. charts) for doses of cyclophosphamide or rituximab.
- For steroids, the key features to document are the start and stop dates (ie. need to input highest dose [start and stop date], lowest dose [start and stop date]).

- **INDUCTION**
  - First 3-6 months
  - Includes:
    - IV methylprednisolone (=solumedrol)
      - Usually 500mg x first 3 days
      - Input into 'Treatment – intermittent pulse admin'

    - PO prednisone
      - Follows on from IV methylprednisolone
      - Usually starts 1mg/kg/day (max 60mg) and tapers down over weeks/months and often continues into maintenance phase at 5-10mg daily. However, new evidence from the PEXIVAS trial has a more rapid steroid taper associated with less side effects but equal efficacy, and therefore this will become common practice with time.
      - Input into 'Treatment – continuing meds'
      - IMPORTANT to keep track of dose changes (will need to review clinic letters to input changes since last distiller update)

    - IV cyclophosphamide (CYC, 15mg/kg, adjusted for age/creatinine)
      - Usually weight based dose x 6, every 2-3 weeks
      - Input into 'Treatment – intermittent pulse admin'

    - PO cyclophosphamide (2mg/kg/day)
      - Instead of IV CYC
      - Daily from initial presentation
      - Input into 'Treatment – continuing meds'
      - IMPORTANT to put stop date when switching to maintenance (i.e. Usually azathioprine)

    - IV Rituximab
      - Usually instead of CYC, with steroids
      - Either 375mg/m^2 weekly x 4 weeks
      - OR 1g x 2, fortnight apart
      - Input into 'Treatment – intermittent pulse admin'

    - Plasma exchange (=PLEX)

- Usually between 5-7 sessions
- Usually type = filter
- Input total number of sessions into 'Baseline characteristics – Vasculitis'
- With the findings from the PEXIVAS trial, plasma exchange will largely be reserved for treatment of Anti-GBM disease going forward

- Occasionally methotrexate (MTX) or mycophenolate mofetil (MMF) may be used as induction in mild disease (if date of treatment onset correlates with initial presentation)

- **MAINTENANCE**
  - Usually starts at 3-6 months, once remission achieved
  - Includes 1 or more of:
    - Azathioprine (AZA)
      - 2mg/kg/day, daily
    - Mycophenolate mofetil (MMF) is alternative, PO, daily
    - PO prednisone (as above)
      - Input ALL into 'Treatment – continuing meds'