DeCompress

Project Handbook

Supported by:

HRB Health Research Board

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Part A. Study Introduction

A.1 Abbreviations

ANCA	Anti-Neutrophil Cytoplasm Antibodies
AAV	ANCA-Associated Vasculitis
CKD	Chronic Kidney Disease
COVID-19	Coronavirus Disease 2019 (SARS-CoV-2 infection)
CPL	Central Pathology Laboratory, St James Hospital
CRF	Case Report Form
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form (REDCap)
EDTA	Ethylene Diamine Tetra Acetic Acid
GCP	Good Clinical Practice
GDPR	European General Data Protection Regulation
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PI	Principal Investigator
RDF	Resource Description Framework
REDCap	Study eCRF
RKD	Rare Kidney Disease
RN	Research Nurse
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SJH	St. James' Hospital
SLE	Systemic lupus erythematosus
SOP	Standard Operating Procedure
TCD	Trinity College Dublin
TTMI	Trinity Translational Medicine Institute
VINE	Vasculitis Ireland Network
WHO	World Health Organisation

A.2 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 and transfer from local sites.

A.3 Rare Kidney Disease & Vasculitis Group Statement

All research conducted by and data arising from the DeCOmPRESS study is supported by the Vasculitis Study Group and Rare Kidney Disease (RKD) Biobank. DeCOmPRESS processes should derive in the first instance from the RKD biobank protocol. The DeCOmPRESS study will adhere to the ethical and scientific quality standards of Good Clinical Practice (GCP). The RKD Protocol provides reference and comprehensive 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 European General Data Protection Regulation (GDPR).
- Studies will be conducted in compliance with the RKD biobank study protocol, which will have been authorised by the appropriate 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.4 Study Contact Details

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CPL Chief Medical Scientist
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A.4.1 Local Study Site Contacts

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A.5 Study Overview

A.5.1 Project Summary

The DeCOmPRESS study will rapidly inform management of immunosuppressed patients who contract Coronavirus Disease 2019 (COVID-19) by defining the natural history and immunological manifestations of the disease in these patients. We aim to determine whether immunosuppressant therapy for chronic autoimmune disease protects against the cytokine storm associated with COVID-19 and reduces the severity of the clinical syndrome, thereby paradoxically improving rather than worsening clinical outcome. We shall achieve this by studying a large existing cohort comprising 70% of patients with systemic vasculitis in Ireland. 850 patients have provided consent for periodic blood sampling and data collection for investigation of infection in vasculitis. Of these, we estimate that we will be able to sample 306 who develop COVID-19 over the next year (153 in the next 3 months). We will obtain a granular clinical dataset, which will be linked to an existing clinical phenotype and blood samples analysed by flow cytometry and ELISA to define the immunophenotype and cytokine profile. Results will be rapidly disseminated to relevant stakeholders. Importantly, use of a FAIR COVID-19 dataset (designed to be interoperable with international data collection initiatives) and deposition of data in an open science repository will allow ready integration with other studies to maximise the impact of this project. Thus, this study will rapidly deliver critical information on how patients with autoimmune and chronic inflammatory diseases should be managed during this pandemic.

A.5.2 Project Background

The course of SARS-CoV-2 infection in patients with systemic autoimmune diseases who take immunosuppressive medication, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and Anti-Neutrophil Cytoplasmic Antibody (ANCA)-Associated vasculitis (AAV), is almost completely undefined. Official guidance to these patients who develop COVID-19 is speculative, modelling of their natural history is ill-informed and clinical management relies upon generic approaches to cutting immunosuppression and providing “stress dose” corticosteroids. However, early evidence indicates that the most damaging facet of COVID-19 is a dysregulated “cytokine storm” (Figure 1), suggesting that immunosuppression (especially cytokine-blocking medications such as tocilizumab) may have both good and bad effects. Indeed, previous data suggest that biologic immunosuppressant agents might protect against severe sepsis (OR=0.56). Additionally, these patients often have disease-associated co-morbidity, including kidney failure, and many are elderly. If concurrent immunosuppressive therapy is viewed as an additional vulnerability factor, rather than as protective, then a ward-based ceiling of care might erroneously be applied. We do not currently know whether SARS-CoV-2 infection is more or less severe in immunosuppressed patients.

We will address this unmet need and contribute to the national and global COVID-19 response by analysing the impact of immunosuppression on the natural history of, and immune response to, SARS-CoV-2 infection. We will use systemic vasculitis as a prototypical chronic autoimmune disease requiring long-term immunosuppression; however, findings will be broadly revealing about other chronic autoimmune diseases and will inform how patients should be managed during this pandemic.

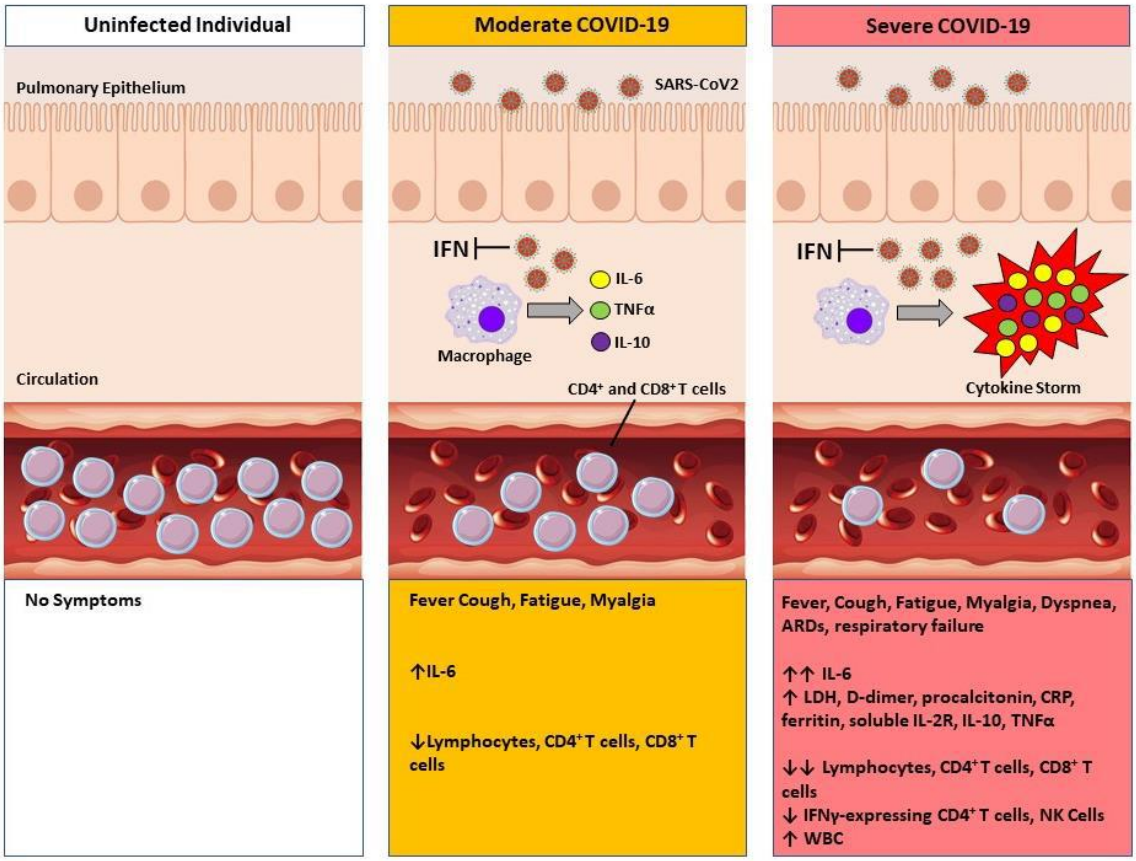

Figure 1: Immunopathogenesis of COVID-19

A.5.3 Study Population

The RKD Biobank cohort comprises over 850 members with systemic vasculitis. This bioresource, which includes 70% of Irish AAV cases, has already supported several published studies. All recruits have provided biological samples during periods of remission and have consented to further sampling and data collection as envisaged in this project. All patients with systemic vasculitis enrolled in the RKD registry developing COVID-19 over the next 12 months will be recruited to the DeCOmPRESS study as described in Section B.2 below.

Based on epidemic modelling we estimate that 60% (n=510) of our cohort will become infected with COVID-19 over the next year (50%, n=255 over next 3 months). Of these infected patients 80% (n=408/204) will be symptomatic. We aim to recruit 75% (n=306) of these cases. Based on preliminary data (current immunosuppression exposure in our cohort), we expect that two thirds (n=202) will be exposed (immunosuppressed)

and one third (n=104) will not. We expect 52% of cases in the non-immunosuppressed group will meet our definition of severe infection. This expected sample size will have 80% power to detect an OR=0.5 (i.e. absolute frequency of severe infection of 35% in immunosuppressed group) or smaller, with 2-tailed alpha of 0.05.

A.5.3.1 Patient Group – COVID+ Vasculitis with Immunosuppression

The patient group for this study comprises RKD Biobank recruits with a diagnosis of systemic vasculitis as defined by the RKD protocol, and diagnosis of COVID-19 as confirmed by polymerase chain reaction (PCR) and/or retrospectively by serology. Patients with a high clinical suspicion of COVID-19 but no timely PCR test should be included pending definitive antibody testing. Review of REDCap will be used to determine type, use, dosage, and frequency of immunosuppressive medications for subsequent analysis. Immunosuppressive medications are defined as >5mg prednisolone, azathioprine, mycophenolate or methotrexate currently; rituximab or cyclophosphamide within previous 6 months. See Appendix II for full details of various treatment regimes.

A.5.3.2 Control Group – COVID+ Vasculitis without Immunosuppression

The control group for this study comprises RKD Biobank recruits with a diagnosis of systemic vasculitis as defined by the RKD protocol, and diagnosis of COVID-19 as confirmed by PCR and/or serology. Patients with a high clinical suspicion of COVID-19 but no timely PCR test should be included pending definitive antibody testing. Subsequent review of REDCap will be used to determine type, use, dosage, and frequency of immunosuppressive medications for analysis. Approximately a third of patients in the RKD Biobank are receiving minimal immunosuppression, defined as ≤5mg daily prednisolone, and no other immunosuppressants (including no cyclophosphamide or rituximab within the last 6 months). These patients will act as our control group and appropriate statistical methods will be employed to account for the effects of treatment on their disease status.

Additional controls may include:

- COVID-19- RKD biobank recruits, comprising those recruited based on clinical suspicion and no PCR test, and who subsequently test negative on an antibody test.
- COVID-19+ patients without immunosuppression and non-vasculitis autoimmune disease; these may be derived from collaborative work with other clinical partners, including rheumatologists caring for patients with rheumatoid arthritis.
- COVID-19+ patients with immunosuppression and non-vasculitis autoimmune disease; these may be derived from collaborative work with other clinical partners.
- Matched archived Patient Group samples from periods of remission/active AAV; these samples will be tested for SARS-CoV-2 antibodies and serum cytokine levels.

- COVID- Healthy/Disease controls as defined by the RKD Biobank criteria; these samples will be tested for SARS-CoV-2 antibodies and serum cytokine levels.

See Appendix II for full details of various treatment regimes.

A.5.4 Study Design

This is a prospective cohort sub-study of the RKD biobank with collection of clinical data and biospecimens as defined in the primary RKD protocol. The primary objective of this study is to determine if the disease course of COVID-19 is more or less severe in patients with autoimmune disease receiving immunosuppressive therapy. Our hypothesis is that COVID-19 is less severe among immunosuppressed patients due to blunted cytokine storm. The aims of the DeCOmPRESS study are:

- Characterise immune cell subsets altered in COVID infection and compare differences in immunosuppressed persons (see Table 1);
- Profile serum cytokines in COVID infection and examine differences in immunosuppressed patients (see Table 1);
- Use clinical data to track differences in immunosuppressant use and outcomes relating to COVID-19;
- Use advanced statistical methods to combine these data and effectively report outcomes;
- Determine whether immunosuppressant therapy for chronic autoimmune disease protects against the cytokine storm associated with COVID-19 and reduces the severity of the clinical syndrome.

A.5.5 Research Timeline

The proposed start date for this project is June 1st 2020 with an estimated timeframe of 18 months. We will produce a preliminary research report at 3 months (September 1st) and a final report at 18 months. Existing infrastructure supports the immediate implementation of this work. Our aim is to recruit 306 COVID+ vasculitis patients by the 18-month deadline, with a target cohort of 153 recruits at 3 months.

Table 1: Surface Markers for Immunophenotyping of Leukocyte Subsets and Circulating Cytokines

T/B/NK Cells	CD45, CD3, CD19, CD56, CD16, TruCount Beads
Naïve/Effector T Cells	CD27, CD197, CD45RA
Activated T Cells	CD38, HLA-DR
Naïve/Memory/Class-Switched Memory B Cells	CD27, CD45RA
Transitional B Cells	CD38, CD24
$CD21_{low}$ B Cells	CD21
Classical/Intermediate/Non-Classical Monocytes	CD14, CD16, HLA-DR
Neutrophil Activation	CD10, CD16
Cytokines and Circulating Markers	IL-1β, IL-6, IL-8, IL-10, TNFα, IL-12p70, sCD25, LDH

A.5.6 Study Methodology

A.5.6.1 Patient Identification

Patients with vasculitis previously recruited to the RKD Biobank will be identified, where possible, at the time of COVID-19 diagnosis and before induction treatment has been initiated. Patients will be treated at the local inpatient site and clinical data recorded in REDCap. This requires an efficient and responsive workflow to allow recruitment at short notice and outside office hours. Patients may be identified sometime after resolution of COVID-19; immunophenotyping will not be possible in these cases.

A.5.6.2 Study Samples

Serum and plasma samples will be obtained and transported using the standard RKD protocol. The samples will be transported to the Central Pathology Laboratory (CPL) at St. James' Hospital (SJH) for immunophenotyping analysis (see Table 1). Fresh whole-blood samples will be analysed by flow cytometry and serum will be archived to allow subsequent batch measurement of cytokines in historical (obtained during stable remission), active infection, and convalescent samples. A simplified workflow for this process is summarised below and demonstrated in Figure 2.

1. Identification of a vasculitis patient (RKD Biobank recruit) with a diagnosis of COVID-19 at one of the participating sites (Section B.2).

2. RKD ID is communicated to the Project Manager to allow co-ordination of sample transport (Section B.3.3).
3. Sample collection is organised and serum and plasma samples are drawn a maximum of 4 hours ahead of scheduled sample collection (Section B.3).
4. Samples are transported to the CPL at St. James' Hospital, where they are processed and stored (Section B.3.4).
    - Note: maximum 24 hours from sample collection to processing – optimum <4
5. Local Research Nurse enters clinical data into the REDCap database and COVID-19 instrument (Section B.4.2).
6. Optional: Clinical data, immunophenotyping data and patient reported symptoms and outcomes from patientMpower app are integrated using ADAPT RDF data engine (Section B.6).
7. Once a reliable serological test for prior COVID-19 infection is available, all subjects will be tested using convalescent serum to confirm immunity to SARS-CoV-2, which will also identify mild/asymptomatic cases (Section B.5).
8. Data will be reported and disseminated to relevant stakeholders at regular intervals (Section A.5.8).

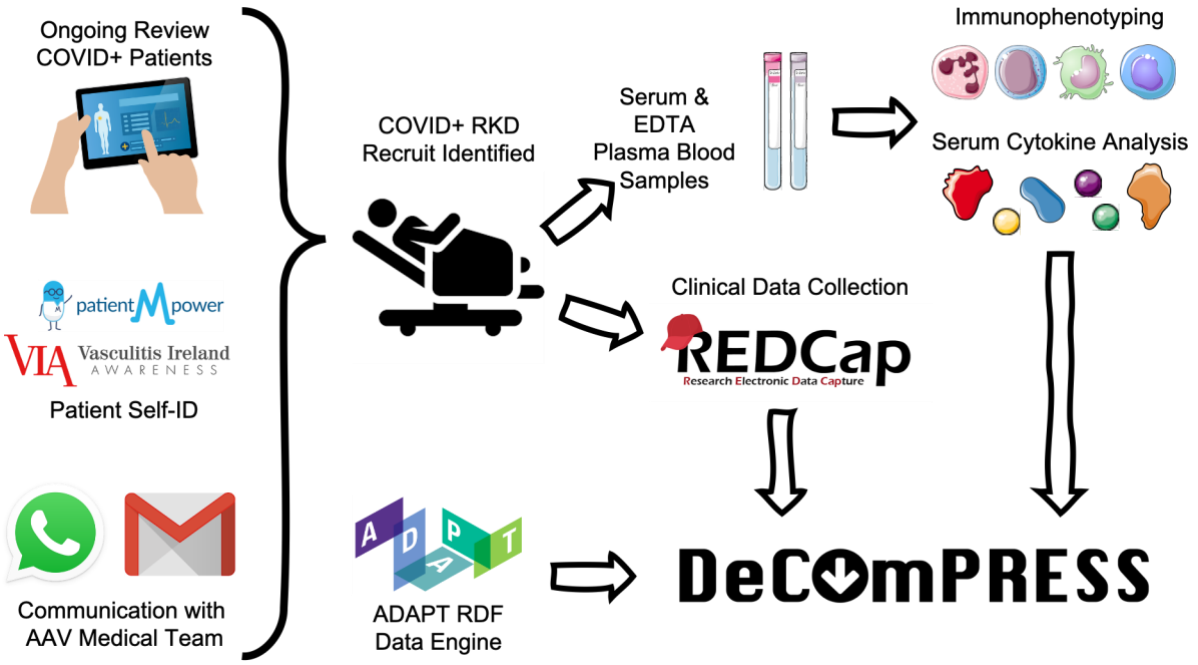

Figure 2: DeCOmPRESS Study Design Schematic

A.5.7 Data Outputs

Our key outcome measure is severe COVID-19 infection, defined as **death, ICU admission and/or need for high-flow oxygen/non-invasive ventilation**. We will record data from a variety of sources, including;

- Detailed clinical information from historic RKD database and COVID-19 REDCap instrument
- Blood samples (historic from archived biobank samples, during infection, convalescent)
    - Immunophenotyping data (see Table 1)
    - Serum cytokine measurements
    - SARS-CoV-2 serology
- PatientMpower app-derived patient-reported symptoms and quality of life measurements

Data analysis will employ the ADAPT Resource Description Framework (RDF) data integration engine and multivariable logistic regression with adjustment for demographics, comorbidities, and vasculitis disease activity. Exploratory analyses include (but are not limited to):

1. Patient-reported symptom profile (immunosuppressed vs. not)
2. Immunologic cytokine profiling and measurement of serologic response to infection (immunosuppressed vs. not)
3. Sub-group analyses comparing induction vs. maintenance immunosuppression, rituximab vs. cyclophosphamide induction.
4. External comparison to COVID-19 patients without vasculitis or immunosuppression.

A.5.8 Data Dissemination

A fundamental requirement of the DeCOmPRESS study is the timely and open-access dissemination of study results. We will produce a preliminary research report at 3 months and a final report at 18 months. The Project Manager and PI will be responsible for study reporting. Audits of data access will be a fundamental part of this reporting procedure. All research outputs will be reported in real time in open access formats and shared with the relevant stakeholders in line with the HRB Joint statement on sharing research data and findings relevant to the novel coronavirus (COVID-19) outbreak. This study protocol will be published on the HRB open research once compete. Additional information can be found in the DeCOmPRESS Data Sharing Plan.

A.6 Ethical Considerations

DeCOmPRESS procedures are incorporated into the RKD ethical framework, with approval obtained in all RKD recruiting sites. All participants have provided consent for research projects deriving from the samples and clinical data. The DeCOmPRESS project has been approved by the RKD Steering Committee. This project has received approval from Chairman of the SJH/Tallaght Ethics Committee (REC: 2020-04 List 13 – Amendment (22)). The Health and Safety Authority has directed all university SARS-CoV-2 work to happen at containment level 3. Non-propagative work in clinical labs can proceed at containment level 2, and DeCOmPRESS patient samples will be

processed in the CPL at St James's Hospital with the appropriate protective measures (see Section B.3.4). Additional validation experiments will be carried out in TTMI when the appropriate safety measures are in place.

Part B. Study Procedures

B.1 Informed Consent

All patients previously enrolled to the RKD Biobank have already provided informed consent for their data and specimens to be used in studies like DeCOmPRESS. Full details are provided in the RKD Biobank protocol. Patient information leaflets are available to download at: https://www.tcd.ie/medicine/thkc/research/rare.php. In cases of COVID-19 in a patient with vasculitis who has not been recruited to the RKD biobank, standard recruitment procedures should be followed.

B.2 Study Incorporation Procedure

Exact sources and procedures will vary by local study site. COVID-19 patients who are currently enrolled in the RKD Biobank should be identified and their COVID diagnosis reported as soon as possible to the "Acute Vasculitis" WhatsApp group, ensuring that no identifiable features are disclosed. *This is the most efficient way to notify the DeCOmPRESS team.* The treating physicians should make the patient's local vasculitis physician aware of any confirmed cases as soon as possible. The local recruiting doctor/Research Nurse/medical team should also review local COVID cases regularly to identify additional vasculitis patients/RKD recruits. The standard procedure is summarised below and in Figure 2.

There are 2 key ways to identify suitable subjects for the DeCOmPRESS study:

1. Actively upon admission for COVID-19 treatment.
    a. RKD Biobank recruits with confirmed COVID-19 diagnosis will be identified by their local medical team.
    b. For COVID-19 patients presenting with their first acute vasculitis encounter please recruit to RKD Biobank as per protocol (https://www.tcd.ie/medicine/thkc/research/rare.php).
2. Retrospective review of local COVID-19 admissions and PCR testing for RKD recruits.
    a. Daily admissions with COVID-19 will be screened by the local research nurse for RKD recruits.
    b. Hospital SARS-CoV-2 PCR data can also be reviewed periodically to identify RKD recruits that may have been missed at the time of testing.

Upon identification of a suitable DeCOmPRESS study subject follow the steps below:

1. Confirm Main Study ID prior to the collection of clinical data or any study samples.
2. Notify the research team of potential study recruits (identified by their Main RKD Study ID) via the "Acute vasculitis" WhatsApp group.

   a. Alternatively contact Prof. Little (mlittle@tcd.ie or 086-609-6068) or the Project Manager (Emma Leacy, leacyej@tcd.ie or 085-748-4173) directly
   b. **Important:** Do not include any patient identifiable information in the message.
3. Confirm an appropriate time for sample collection and transfer with the Project Manager. This will ideally be in the morning (before 12pm) to allow for efficient processing.
4. Explain to the patient that additional blood samples will be taken for research use in accordance with the RKD Biobank protocols.
5. Collect samples and complete REDCap COVID instrument as specified in Section B.4.

B.2.1 Ongoing Review of COVID+ Patients at Study Sites

To reduce delays in the AAV medical team becoming aware of COVID+ RKD recruits, COVID cases at registered study sites will be cross-referenced with RKD recruits at those locations. A retrospective review of COVID-19 admissions at each hospital site should be carried out at the start of the DeCOmPRESS study to confirm if any RKD recruits have contracted COVID-19 and are suitable for inclusion. Subsequent weekly checks of COVID-19 admissions should be completed. The local Research Nurse at each site is responsible for systematically reviewing these cases and notifying the Project Manager.

B.3 Sample Collection

The RN/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. The RKD protocol should be followed; the relevant procedures are appended here for ease of use. Note that the Biomnis courier company require up to 24 hours' notice so, in general, the sample should be obtained *with morning bloods on the day after recruit identification*.

B.3.1 Summary of Order of Blood Sample Collection (as per RKD protocol)

NOTE: Cap colours may vary by site.

1. 1x 5ml serum tube (usually red cap)
   - Storage: room temperature
   - Contains clotting activators to encourage the clotting process and enable collection of serum 30 minutes after collection.
2. 1x 5ml EDTA tube (usually purple cap); room temperature

- Storage: room temperature
- EDTA = ethylene diamine tetra acetic acid
- A colourless crystalline acid that acts as a strong chelating agent.
- Forms an anticoagulant sodium salt used to keep blood samples from clotting before tests are run.
- Can be used to collect blood for DNA extraction, PBMC preparation and plasma.

B.3.2 Procedure for Blood Sample Collection (as per RKD protocol)

Note: Cap colours may vary by site.

1. All sample containers and equipment should be assembled prior to the procedure.
2. 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.
3. Position the patient safely and comfortably in a chair/couch, ensuring that the arm is in the correct position to draw blood.
    a. Ensure that the patient's mouth is free from any food or gum. Collect 5ml of blood directly into a vacutainer blood collection tube containing **serum clot activator (red top),**
    b. Collect 5ml tube of blood into a vacutainer blood container containing **EDTA (purple top) for plasma and DNA**,
2. 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**.**
3. 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.**
4. 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.
5. Write the time of collection on each tube.
6. Print the Main RKD Study ID on each tube.
7. Store both tubes at room temperature.

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.3.2.1 Sample Labelling

The key identifier for patient samples is the RKD Biobank Main Study ID.

Important: When transferring patient samples to CPL, 'Location 553' and 'Decompress Study' should also be included in printed sample labels on the outer packaging.

Additional notes such as “for Immunology LABMED St. James” and “Please alert Immunology on arrival in SJH” should be printed and attached to the outer packaging. We recommend a sturdy envelope or container for the samples.

B.3.3 Sample Transfer

Once samples have been taken please contact the Project Manager to arrange transfer of samples.

- All samples are transferred as soon as possible after sampling to CPL at St. James’ Hospital for processing.
    - Upper limit from sample collection to laboratory analysis is 24 hours
- Samples should be double bagged and clearly labelled with study ID.
- Samples should be left at main hospital reception or another clearly defined and accessible location.
- Samples will be transferred by a member of the DeCOmPRESS study team (to reduce arm to processing time for these samples) OR collected by a courier.
    - Courier will be organised by the Project Manager or Biobank Technician. The courier company will use category A packaging to transport the samples.
        - Sample transport is fulfilled by Biomnis Ireland via the Trinity BioBank (TCD) account.
        - Contact Biomnis by phone (1800-252-967) to arrange an appropriate time for sample transfer.
        - A Booking Form with contact details of local RN must be completed in full and sent to logistics@biomnis.ie.
            - See https://www.tcd.ie/medicine/thkc/research/rare.php
        - For full details of courier sample transfer procedure please contact the RKD Biobank Co-ordinator.
- Samples are left in a container in the vestibule entrance to CPL.
    - Reception staff will be notified to bring these samples to the attention of Immunology so that they come directly into the laboratory and are not opened at Specimen Reception
- Receipt of samples by CPL should be confirmed via the “Acute vasculitis” WhatsApp group
- Samples will be processed according to the CPL Immunophenotyping Procedure described in Appendix III.

B.3.4 Immunophenotyping Procedure

Surface markers for immunophenotyping of leukocyte subsets and circulating cytokines to be measured in this study are listed in Table 1. Samples analysed by CPL

are recorded in the TELEPATH System and assigned a unique Laboratory Number linked to the RKD study ID (as printed on the sample tubes). This number is used by CPL for sample identification, sample naming and storage. The full immunophenotyping procedure is detailed in Appendix III.

B.3.4.1. Data Transfer from CPL

Immunophenotyping and serum cytokine data will be exported to a dedicated OneDrive Folder accessible only to CPL staff, the Chief Investigator and the Project Manager. Data will be transferred as results become available. Regular audits of data will be performed by the Project Manager as defined in the Data Management Plan.

B.4 Patient Data Collection

The local RKD recruitment log contains patient identifiable information and is a link between an individual and their study ID. This data is 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 study findings. A central, non-identifiable record is also maintained by the Project Manager.

B.4.1 Confidentiality

The Main RKD Study ID should be recorded on all study related documents and files. This serves 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.4.2 eCRF (REDCap) Completion Guidelines

Anonymised patient data should be recorded electronically in the eCRF (REDCap). Registration fields are referred to as ‘instruments’ in REDCap. A new detailed COVID-19 instrument has been added which includes information such as symptom onset, diagnosis, and medications. Efficient tracking of presence and type of immunosuppression are key to this study. Comprehensive guidelines for completing the REDCap COVID-19 instrument are available in the RKD Clinical Data Entry SOP and summarised in Section B.4.2.1 below. This entry should be completed as soon as possible after patient recruitment to the study. Particular attention should be paid to the COVID-19 instrument and medication entries (which allows accurate assignment to immunosuppressed or non-immunosuppressed groups). A single COVID-19 instrument is entered and is linked to a standard encounter entry. It may be necessary to return later to complete some fields if the patient’s clinical episode has not been completed.

The Project Manager will contact the recruiting doctor/Research nurse/medical team no more than 7 days after sampling to confirm REDCap data completion. It is the

responsibility of the research nurse and local lead investigator to ensure that all steps are completed competently. It may be necessary to consult with the patient's medical doctor in order to accurately complete the registry entry. If in doubt, don't enter the data, check with the doctor. Timely and accurate completion of REDCap data is essential to this project

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 their privacy and confidentiality. Data recorded in the eCRF should be consistent with the source documents.

All participants in the DeCOmPRESS study are existing recruits to the RKD Biobank. Patients will be classified as to either "Patients" or "Controls" based on immunosuppressant use (see Section A.5.3). Appropriate patient grouping will be confirmed after completion of REDCap medication fields (see Sections B.4.2.1.2 and B.4.2.1.3). Therefore, is it essential that REDCap data are thoroughly and correctly completed.

B.4.2.1 eCRF (REDCap) Completion Protocol

B.4.2.1.1 General REDCap Navigation

- Pre-determined users can access REDCap through the following link: https://rkd.tchpc.tcd.ie/
    - Study PI is responsible for granting access permissions
    - The project title is "Rare Kidney Disease - RIT Production - 03/09/19"
- In the Data Collection menu on the left panel select 'View / Edit Records'
- To search for a particular RKD ID, use the 'Choose an existing RKD ID' dropdown.
    - Records can also be identified by searching date of birth, hospital or any other field, by using 'Choose a field to search'
- Click on 'Edit Records' from the left-hand shortlist to edit any RKD ID record.
    - The various instruments can be navigated from this shortlist.
- Always **save** each page prior to moving to a different instrument. Options include:
    - **'**Save & Stay'
    - 'Save & Exit Form'
    - 'Save & Add new instance' (e.g. to add another drug in treatment instruments)
- When an instrument is fully completed, change 'form status' to 'complete' – this will change the red icon to green on the shortlist.
- **Exports** can be customised using the 'Data Export' tool. Data can be exported to various programmes including Excel and R. Basic statistics and filtering options are also possible. Further guidance is provided in the support videos in REDCap or from the TCD HPC Department.

B.4.2.1.2 Treatment Instrument: Continuing Medications

- Document maintenance immunosuppression (e.g. Azathioprine or Rituximab) and steroid doses, with start and end dates (see appendix VIII for treatment explanation).
- Where possible, aim to document the steroid wean. Practically, one should prioritise the start and end dates for the highest and lowest (this could be 0 mg, i.e. cessation) steroid doses.
- Select the drug from the dropdown list. If it is not listed, select 'other' and proceed to type the drug name in the field 'Drug, ATC', which is backed by the ATC ontology.

B.4.2.1.3 Treatment Instrument: Intermittent Pulse Administration

- Document each dose and date of intravenous therapy, including: methylprednisolone, cyclophosphamide and rituximab.
- To add a new instance, click on the arrow beside 'current instance':
- Select the drug from the dropdown list. Select 'other' if the drug is not listed to enter it into the ATC ontology backed field.

B.4.2.1.4 COVID-19 Instrument

The COVID-19 specific instrument was modelled on the current 'encounter' instrument used for clinic visits. The information will mandate a parallel encounter (as a pop-up within the COVID-19 instrument) and other relevant fields (via piping). Detailed information about the COVID-19 instrument can be found in the document "RKD Vasculitis COVID-19 Module" on the DeCOmPRESS webpage (https://www.tcd.ie/medicine/thkc/decompress/).

- This is a critical section and should be completed ASAP after COVID-19 diagnosis.
- Fields below are presented as they appear in the COVID-19 Instrument
- Sections to be completed are as follows:
    1. Reporter Information
        a. Name of physician providing care for vasculitis patient
        b. Name of centre providing care
        c. Date this form completed
        d. Please select all databases this form has been shared with
    2. Patient Information (Part 1)
        a. Country of residence
        b. County of residence
        c. Education
        d. E-cigarette or Vape use
    3. Patient Information (Part 2)
        - All fields are obtained from existing fields/encounters. It is important these fields are completed in the relevant sections to ensure an enriched dataset.

4. ESKD and VDI Score
    a. End-stage Kidney Disease (ESKD)
        i. Date of End-stage Kidney Disease (Date of commencement on dialysis or transplant, whichever first)
        ii. Type of Renal Replacement Therapy (RRT)
    b. VDI Score

Generate a new encounter instrument by clicking 'New Encounter Instance' within the COVID-19 instrument, ensuring that the encounter date matches the date of sample collection.

- Select 'yes' for 'COVID-19 related entry'
- Click 'Save & exit form' at bottom of pop-up after completing all fields listed below.

1. Vasculitis/Disease status at time of C-19 diagnosis
    a. Employment status
    b. Disease activity at time of C-19 symptom onset or diagnosis if asymptomatic (physician global)
    c. Urinalysis Done
        i. Urinalysis Protein
        ii. Urinalysis Blood
    d. Last eGFR (CKD-EPI) prior to C-19 diagnosis
        i. This field is obtained from the preceding encounter for the bespoke export. No additional data entry is required.
    e. Dialysis dependent
        i. Date of dialysis start +/- stop
    f. Weight (kg)
    g. Height (m)
    h. BMI
    i. Clinical samples obtained
    j. BVAS Scoring
    k. BVAS Score
    l. Do you think vasculitis is relapsing in this encounter?
        i. Physician's assessment at time of clinical review.
    m. Adjudicated probability of relapse?
        i. This field is completed retrospectively by senior clinician, taking all variables into account (e.g. clinical signs/symptoms, laboratory values, biopsy, etc.)
2. Medication

- Includes any medication the patient is on within 2 weeks of COVID-19 diagnosis
- All questions relate to drugs/doses at time of C-19 symptom onset (or diagnosis if asymptomatic)

- **Please ensure 'Treatment - continuing medication' AND 'Treatment – intermittent pulse administration' is up to date in terms of dose and start/stop dates for all immunosuppressive medications**.
    a. Immunosuppressive status
        i. *If Immunosuppressive status* ≠'Treatment Naïve'
    b. Corticosteroids
        i. Current corticosteroid dose
    c. Corticosteroids in response to this clinical encounter/episode
        i. If Immunosuppressive status ≠'Treatment Naïve'
    d. Immunosuppressive medication
        i. Patients may be on >1 Immunosuppressive agent. If so, select a second agent from the field 'Additional Immunosuppressive medication'*.*
        ii. Consequently, complete whether this additional agent was increased / no change / reduce, etc.
        iii. If patient is not on any additional agents, leave blank.
    e. Immunosuppressive medication in response to this clinical encounter/episode
        i. If Immunosuppressive medication in response to this clinical encounter/episode = Increased: Response to increased immunosuppression
    f. Angiotensin-converting-enzyme inhibitor at C19 diagnosis (ACE-i)
    g. Angiotensin II receptor blocker at C19 diagnosis (ARB)
    h. Non-steroidal anti-inflammatory drug at C19 diagnosis (NSAID)

3. COVID-19 Questions; note that, for measurement of clinical parameters please record the peak/nadir value from the entire COVID-19 episode
    a. Date of C-19 symptom onset (if known)
    b. Date of C-19 diagnosis
    c. Interval (days) between symptom onset and diagnosis (if known)
    d. Age at C-19 diagnosis (years)
    e. Location at which C-19 diagnosis was made
    f. Method of C-19 testing (select the most objective option)
        i. Level of SARS-CoV-2 (COVID-19) PCR
    g. SARS-CoV-2 (COVID-19) IgM
    h. SARS-CoV-2 (COVID-19) IgG
    i. Admission to hospital required
        i. Date of admission
    j. Admission to Intensive Care Unit
        i. Date of admission
    k. Interval (months) from AAV diagnosis to C-19 diagnosis
    l. Have patient's symptoms resolved at time of this report?
        i. Date of symptom resolution (if known)
    m. Interval (days) between symptom onset (if known) and symptom resolution OR current date (if symptoms persist)

n. Date of hospital discharge (if known)
o. Length of stay (days)
p. Infection Acquisition
q. Clinical features at outset (check all that apply)
r. Body temperature (highest recorded, °C)
s. CRP (mg/L)
    i. *Please enter the highest/peak recorded lab value for each field*
t. Creatinine (mmol/L)
u. AST (U/L)
v. ALT (U/L)
w. Haemoglobin (g/dL)
x. Total White Cell Count x 10^9/L
y. Neutrophil count x 10^9/L
z. Lymphocyte count x 10^9/L
aa. Neutrophil / Lymphocyte ratio
bb. Urine Protein Creatinine ratio (uPCR, mg/mmol)
cc. ANCA IF
dd. Anti-PR3 level
ee. Anti-MPO level
ff. Platelet count x 10^9/L
gg. Creatinine kinase (U/L)
hh. D-dimer (mg/L)
ii. Ferritin
jj. Lactate (mg/dL)
kk. Prothrombin time (s)
ll. Lactate dehydrogenase (U/L)
mm. Troponin
nn. Troponin Units
oo. Findings on chest imaging
pp. Were antibiotics administered?
qq. Was treatment administered for C-19 infection (other than best supportive care)?
rr. Complications / Disease Course (check all that apply)
    i. If AKI; please complete encounter pop-up.
    ii. If Dialysis dependent; Date of dialysis start +/- Date of dialysis stop
ss. Concomitant respiratory pathogens detected (select all that apply):
    i. Indicate the type of Infection (in addition to concomitant respiratory viral pathogens)
    ii. Select the microorganism (if known)
tt. C-19 Outcome (Select the highest level of support the patient received)
    i. If Death; Date of death, Cause of death
uu. May we contact you to get more information about the outcomes of this case?
vv. Would you like to share any lessons or other aspects from this case?

i. Please include as much information as desired, this will greatly help patients and colleagues.

- Additional fields obtained from existing encounters:
    a. Gender
    b. Date of birth
    c. Ethnicity
    d. Smoking History
    e. Co-morbidities
    f. Vasculitis diagnosis
    g. ANCA serology (at time of diagnosis)
    h. Date of formal vasculitis diagnosis
    i. Biopsy performed
    j. Histologically confirmed diagnosis
    k. Vasculitis diagnosis confidence
    l. Treatment – continuing medications
    m. Treatment – intermittent pulse administration (IV)

B.4.3 Site File Management

All data collected from the DeCOmPRESS study will be stored securely as detailed in the Data Management Plan. All relevant documents and databases will be compiled and maintained by the Project Manager. The key documents generated from this study will comprise:

- A central recruitment log comprising RKD ID, date of recruitment and data collected
- Immunophenotyping data from CPL analysis.
- Patient reported symptoms from patientMpower app.
- The ADAPT RDF Integration Engine exports, which will allow statistical analysis.

These data will be securely stored as specified in Section C below and in the comprehensive Data Management Plan.

B.5 Serological Testing

A standard RKD sample set will be obtained at a routine clinical visit in all RKD recruits during 2020. The serum from this sample set will be analysed in TTMI to assess for the presence of SARS-CoV-2 antibodies.

Section to be updated as final assays validated

B.6 ADAPT RDF Data Integration

Data analysis will employ the ADAPT Resource Description Framework (RDF) data integration engine and multivariable logistic regression with adjustment for demographics, comorbidities, and vasculitis disease activity. Data integrated into ADAPT's analysis networks will be stored using RDF model to facilitate analysis and data sharing. The RDF model stores data in a "triplestore" database. This is a purpose-built database for the storage and retrieval of triples through semantic queries. Semantic triples codify statements about the data in the form of subject–predicate–object expressions. The RDF model facilitates enhanced data analysis and sharing. Data from various sources will be formatted appropriately to allow efficient uplift into the triple store.

Appendices

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).

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.

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.

Table 2: 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)

Appendix II: Explanation of Typical Treatment Regime

Explanations of typical treatment regimens for AAV are described below. The RN may need to request medical records (i.e. charts) to confirm doses of cyclophosphamide or rituximab. For steroids, the key features are the start and stop dates (i.e. need to input highest dose [start and stop date], lowest dose [start and stop date]).

Induction Treatment

- 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 x6, 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 x4 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:
  - Rituximab, usually given every 6 months
  - Azathioprine (AZA)
    - 2mg/kg/day, daily
  - Mycophenolate mofetil (MMF) is alternative, PO, daily
  - PO prednisone (as above)
    - Input ALL into 'Treatment – continuing meds'

Appendix III: CPL Staining Protocol for Lymphocyte, Monocyte & Neutrophil Surface Marker Panels

Reagent Preparation

- Dilute BD FACS Lyse solution 1:10 with distilled water prior to use.
- Keep antibodies should be maintained on a cool pack at 4°C throughout protocol

Equipment and Materials Preparation

1. Turn on BD Lyse Wash Assistant.
   - Fill tanks with fresh dH20 and BD Cell wash.
   - Empty waste if necessary.
2. Label 12x75-mm Falcon tubes for each patient
   a. T cell gating control
   b. T cell Naïve and Memory panel
   c. T cell activation panels
   d. Monocyte panel
   e. Neutrophil panel
3. Make up antibody mixes for each panel as in Table 3 and add to the corresponding tube.
4. Pipette 100µl of blood into each tube and vortex.
5. Run samples on the BD lyse wash assistant.

Table 3 CPL Antibody Mixes for Immunophenotyping

	Antibody	1 sample	Volume to add to each tube
T cell Gating Control	CD45 CD3 CD4 CD8	5ul 5ul 5ul 5ul	20ul
T cell Naïve and Memory Panel	CD45 CD3 CD4 CD8 CD45RA CD27 CD197	5ul 5ul 5ul 5ul 5ul 5ul 5ul	35ul
T cell Activation Panels	CD45 CD3 CD4 CD8 CD38 HLA-DR	5ul 5ul 5ul 5ul 5ul 5ul	30ul
Monocytes	CD45 CD14 CD16 HLA-DR	10ul 10ul 20ul 10ul	50ul
Neutrophils	CD45 CD10 CD16	10ul 10ul 20ul	40ul

BD Lyse Wash Assistant Procedure

1. Incubate samples for 15 minutes in the dark at room temperature.
2. Centrifuge at 500g for 5 minutes and decant.
3. Add 2ml cell wash and mix gently.
4. Centrifuge at 500g for 5 minutes and decant.
5. Add 500µl cell wash and mix gently.

Analyse on the FACS Canto II flow cytometer. Mix samples thoroughly before acquisition.

Table 4 Antibodies and Fluorochromes used for CPL Immunophenotyping

Antibody	Fluorophore	Clone	Company	Catalogue #
CD45	V500-C	2D1	BD Biosciences	655873
CD8	V450	RPA-T8	BD Biosciences	560347
CD3	APC-H7	SK7	BD Biosciences	641415
CD4	PerCP/Cy5.5	SK3	BD Biosciences	332772
CD45RA	PE	-	BD Biosciences	556627
CD27	FITC	-	BD Biosciences	555440
CD197	Alexa Fluor 647	150503	BD Biosciences	560816
HLA-DR	FITC	L243	BD Biosciences	347400
CD38	APC	HB-7	BD Biosciences	345807
CD14	APC	MϕP9	BD Biosciences	345787
CD16	PE	B73.1	BD Biosciences	332779
CD10	APC	HI10a	BD Biosciences	332777

Gating Strategies

Monocyte Gating Strategy

Monocytes are defined as SSCmidCD45+HLA-DR+ and:

- Classical Monocytes: CD14high, CD16−
- Intermediate Monocytes: CD14high, CD16+
- Non-Classical Monocytes: CD14+, CD16+
- Total Monocyte HLA-DR+

Granulocyte Gating Strategy

Granulocytes are defined as SSChigh CD45+ and as:

- CD10+, CD16+

- CD10-, CD16-
- CD10+
- CD10-

Lymphocyte Gating Strategy

Lymphocytes are defined as SSClow CD45+ followed by:

- Naïve CD27+CD45RA+CD4+T cells
- Naïve CD27+CD45RA+ CD197+CD8+ T cells
- CD27-CD45RA+CD197+CD8+ effector T cells
- Activated CD38+CD197+HLA-DR+ CD4 T Cells
- Activated CD38+CD197+HLA-DR+ CD8 T Cells