Joint Research Management Office (JRMO) Research Protocol for Research Studies

Full Title	Inflammation and infective endocarditis
Short Title	Inflammation and infective endocarditis
Sponsor	▪ Queen Mary University of London (Queen Mary) Contact person: Dr Mays Jawad Research Management Office (JRMO) Barts Health NHS Trust & Queen Mary University of London Dept W, 81 Mile End Rd, London E1 4UJ. Phone: 020 7882 7275/6574 Email: research.governance@qmul.ac.uk
IRAS Number ***Integrated Research Application System***	**353031**
Sponsor (EDGE) Number **REC Reference** ***Research Ethics Committee***	Pending
Chief Investigator (CI)	Dr Daniel Harding Clinical Lecturer in Cardiology 07503192714 d.harding@qmul.ac.uk
List of sites	St Bartholomew's Hospital West Smithfield London EC1A 7BE d.harding@qmul.ac.uk
List of laboratories	Biochemical Pharmacology Professor Federica Marelli-Berg f.m.marelli-berg@qmul.ac.uk Charterhouse Square London, EC1M 6BQ
List of technical departments	
List of central facilities	

Contents

2. Glossary

3. Signature page

CI Agreement

The study, as detailed within this Research Protocol, will be conducted in accordance with the principles of Good Clinical Practice (GCP), the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

CI Name: _____

Signature: _____

Date: _____

4. Summary and synopsis

Short title	Inflammation and infective endocarditis
Methodology	Research
Objectives / aims	1. Define the peripheral (blood) and local (valve tissue) immune landscape in infective endocarditis (IE). 2. Confirm that cMET+ T-cells are present in patients with infective endocarditis (peripheral and local) and interrogate their functional role by flow cytometry and RNA sequencing.
Number of participants	100
Inclusion and exclusion criteria	Inclusion: • The principal inclusion criteria is a clinical diagnosis of IE made according to the Duke Criteria. Patients aged 18-85 are eligible. Exclusion: • We will exclude patients with an active solid organ or haematological malignancy as these conditions can influence immune cell frequency and phenotype.
Statistical methodology and analysis (if applicable)	With the support of our local NIHR bioinformatics and bio-repository facility (http://www.whri.qmul.ac.uk/core-facilities/nihr-bioinformatics-and-bio-repository), Multiple parameters will be analysed using multiple group comparisons (ANOVAs) to increase power.
Study duration	18 months

5. Introduction

Background

Infective endocarditis (IE) is challenging to diagnose and manage. Its frequency is increasing as we perform more interventions on patients with pre- existing valve disease2,3. Current strategies are relatively historical; patients require positive blood cultures for a definitive diagnosis and are typically managed with intravenous antibiotics and cardiac surgery4. While the immunological complications of IE are well recognised (e.g. glomerulonephritis, rheumatoid factor positivity), there has been very little research to explore the immune system's role in disease onset and progression5. Our laboratory has discovered and characterised a group of circulating immune cells (cMET+ T-cells) that appear to have a direct role in the progression of another inflammatory heart disease known as myocarditis6. Pilot data (also from our laboratory, n=3 patients) suggests that these cells are detectable in explanted valve tissue from patients undergoing endocarditis surgery. We believe that the presence of these cells in IE implies that there may be a significant contribution to disease progression from the immune system. It is possible that the exposure of cardiac proteins by damage caused by a bacterial infection primes the immune system against self, and that these auto- reactive T-cells then go on to attack and destroy native valve tissue.

Rationale

We would like to investigate whether circulating and valvular cMET+ T-cells are detectable across the wide spectrum of patients with IE and further explore their role in this condition. We are planning to functionally characterise both circulating immune cells and those that are detectable in explanted valve tissue using a combination of flow cytometry and spatial RNA sequencing. We hypothesise that cMET+ T-cells may have utility in the diagnosis of IE and that in patients with a significantly elevated frequency of cMET+ T-cells, we might be able to offer novel (and highly specific) targeted immunological therapy that could improve patient outcomes. To address this hypothesis, we will undertake an observational cohort study in patients with IE to analyse the immune cell status in the peripheral blood and valve tissue (obtained at surgical explant)

Risks / benefits

We do not envisage any risks to this project as we will only be taking blood samples via venepuncture. Only a slight discomfort may be experienced by the patient at the injection site and slight bruising. To minimise bruising, the patient will be advised to apply pressure to the venepuncture site using a cotton swab that will be provided to the patient once the blood has been taken. The patient will also be advised to sit for 10 minutes to ensure that they are feeling well after the blood is taken.

The potential benefit of this projects outweighs the potential risks. The immediate outcomes for this project is to gain a detailed understanding of how the immune system could affect the development and progression of infective endocarditis. This could help to inform our current understanding of why infective endocarditis occurs and help us to develop new treatments.

6. Study objectives

1. We will report a comprehensive analysis of immune cell frequency and function in the peripheral blood and valve tissue from patients with IE. This has never before been performed.

2. We will confirm whether cMET+ T-cells are present in patients with IE (blood and valve), and determine if they may have a diagnostic and therapeutic potential.

3. We will compare immune phenotype during the acute disease phase in IE with convalescence/recovery.

7. Study population

All patients with IE who are managed within the Barts Heart Centre.

Inclusion criteria

- The principal inclusion criteria is a clinical diagnosis of IE made according to the Duke Criteria.
- Patients aged 18-85 are eligible.
- Fluency in English (otherwise interpreters will be used)
- Informed, written consent

Exclusion criteria

- Unwilling or unable to give consent
- Participants who are unable to understand written English
- Patients with an active solid organ or haematological malignancy

8. Study design

This is a prospective cohort study.

Dr Daniel Harding and allocated research nurse (Innocent Bvekerwa) will identify and recruit IE patients whilst inpatients at the Barts Heart Centre (~300 patients/year in this service). Peripheral blood will be obtained at the time of recruitment (DH and IB to perform phlebotomy), valve tissue will be obtained as/when these patients undergo cardiac surgery. Samples will be stored at 4 degrees prior to transfer to QMUL for processing by the scientific team (DH and post-doctoral scientists). Samples will be processed and either stored at -80 for subsequent analysis (blood), fixed in formalin and embedded in paraffin (valve tissue for spatial analysis) or processed and analysed immediately (valve tissue for flow cytometry). 3. Relevant (anonymised) clinical data will be extracted at the point of recruitment (demographic, blood test results, imaging findings) and recorded for downstream correlation analysis. 4. Patients will be followed remotely, and outcome data (death, further surgery, recurrent disease) will be recorded. When the patients attend for their outpatient clinic (in those that survive), they will be offered a further blood test. We will use this sample to perform repeat immune cell analysis in the convalescence phase of their illness. 5. Flow cytometry analysis of immune cells in blood and valve tissue will be performed locally (Biochemical Pharmacology, basement laboratory), whereas spatial RNA analysis will be commissioned from a third party provider (Azenta life sciences).

9. Study procedures

- Laboratory assessments (Please see the laboratories section 12)

- Participant withdrawal (including data collection, retention or replacement for withdrawn participants:

Participants will have the option to submit a written request to be withdrawn from the study at any stage. Participants will also have the option to withdraw consent from further participation in which circumstance no further data will be collected for inclusion. In the unfortunate event that a participant loses capacity, information gathered will continue to be used but no further data will be recorded. The end of the study will be defined by achieving the proposed patient numbers per patient group (outlined above).

10. Statistical considerations

The CI declares responsibility for the statistics and statistical oversight of the study (see CI Signature Agreement section 3).

Sample size and power calculations

The total sample size for this project is 100 patients with IE. We will aim to recruit to 5 IE subgroups (n=20 per subgroup), to reflect the heterogeneous nature of this condition: native valve, prosthetic valve, cardiac-device related, uncorrected GUCH and recurrent IE. Power calculations were performed using G*Power (University of Kiel, Germany) with the support of the QMUL Bioinformatics department. Sample size estimates were based on a Wilcoxon-Mann-Whitney test using the following parameters: $\alpha = 0.05$, $\beta = 0.2$, predicted effect size 0.87. The predicted effect sized used for this power calculation was based on a previously published study comparing the % of peripheral cMET+ T-cells in acute myocarditis and DCM6. Using the above calculation, we determined that a minimum sample size of 10 patients per subgroup would yield statistical significance. To avoid under-powering, we chose to double this target to 20 patients per subgroup.

Method of analysis

1) Flow cytometry of immune cell. Whole blood flow cytometry is a well-established method. Dr Harding's laboratory has access to a flow cytometry core facility, which has machines that can identify up 12 phenotypic markers on any given cell. Moreover, we, have extensive experience in immune cell phenotyping using this method. The key advantages are:
    - It is the gold-standard for immune cell phenotyping
    - A minimal amount of blood is needed (max 200µls; 50µls per patient per antibody panel -see table 3 below)
    - Antibodies are commercially available and have been extensively validated by ourselves and others.

All samples will be processed an analysed within 1 hour of sample collection to minimise degradation of proteins on the surface of the immune cells.

11. Ethics

Application for the current study is pending following submission (IRAS ID 353031).

Informed consent will be obtained and recorded as described as above. Allowances for special groups are detailed above.

The CI will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2013) on research involving human subjects.

The CI will ensure that the study is conducted in accordance with all applicable regulatory requirements which are based on ICH Guidelines for GCP, Trust and Research Office policies and procedures and any subsequent amendments.

The study can only start after approval from a REC, HRA and confirmation of local capacity and capability at each of the participating centres.

Members of the study team do not have any conflicts of interest to declare.

Incidental Findings

Should any incidental findings of clinical significance arise during the course of the study, the participants GP and clinical care team will be informed. Consent to contact the GP will be sought during the enrolment process.

12. Annual Safety Reporting

The CI will send an Annual Progress Report to the REC and the sponsor using the HRA template on the anniversary of the REC “favourable opinion”.

13. Public involvement

There are no UK groups specifically dedicated to raising funds or awareness for infective endocarditis. We have therefore been unable to involve any specific advocacy groups related to this condition in the study design. We do however intend to utilise current public engagement schemes run at the William Harvey Research institute and would also be keen to take part in any events organised by the Barts Charity to help disseminate our findings. Dr Harding has previously been involved in the writing and dissemination of myocarditis patient information resources for Cardiomyopathy UK. We would be very enthusiastic as a team to develop these sort of resources for infective endocarditis and plan to approach Cardiomyopathy UK to facilitate this.We plan to disseminate aspects of our research via newsletters, web-based information, and open forum patient information days. The proposed research will be incorporated into our current public engagement schemes.

14. Data handling and record keeping

Data management

All data generated will be stored within an Excel spreadsheet combining clinical and lab data. The spreadsheet will be password protected stored with a secure hard drive on a protected computer. Access to the password and data will be on a strict need-to-know basis. A full audit trail will be in place.

Source Data

Source data for this study will comprise of a diagnosis of infective endocarditis, which will be obtained from the adult cardiology team, led by Dr Simon Woldman at the Barts Heart Centre. Source data will also be obtained from the laboratory procedures of blood samples taken from patients at the time points mentioned above. Investigators who are directly involved with the study will have access to the source data.

Confidentiality

Dr Daniel Harding, the PI, will ensure only the clinical team has access to patient identifiable information, such as name, date of birth and hospital number. Any data that needs to be sent to the research team at the laboratory at Queen Mary (CI Dr Daniel Harding), a pseudo-anonymised database will be created where each participant will be given a unique study number, ensuring the research team at Queen Mary will not have access to patient identifiable data.

Record retention and archiving

The study data and documentation will be archived in accordance with the relevant regulatory requirements and site SOPs.

A unique alphanumeric patient identifier will be allocated to each participant at the start of the study. Data will be stored using the unique participant identifiers on a secure database. Data will not be stored on personal computers. Confidential documents will be stored in a locked cupboard located in a secure room. Clinical notes will be handled according to trust protocol. The Data Protection Act and the NHS confidentiality code of practice will be adhered to throughout this study. Data may be shared with members of the clinical team to allow ongoing clinical care.

GCP guidelines require that the investigator or the institution maintains all Case Report Forms and all source documents that support the data collected from each participant plus all trial documentation. Measures will be taken to prevent accidental or premature destruction of these documents. Essential documents must be retained for 5 years. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained. If the responsible investigator retires or leaves the institution responsibility for the documentation must be transferred to a person who will accept their custody

15. Laboratories

Central and local laboratories

All laboratory work will be carried out within the Centre for Biochemical Pharmacology, William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ

Sample preparation and collection

Blood samples to assess immune cell phenotypes will be collected from patients who have read the information sheet and signed the consent form. A maximum volume of 15mls will be taken from the patient at any one time and collected into lavender EDTA blood tubes.

Laboratory procedures

The majority of the blood samples will undergo phenotypic analyses using the flow cytometry method. Briefly, 50 microlitres of blood will be labelled with commercially-available antibodies that are conjugated to flurochromes that can be detected by lasers on the flow cytometer. Specialised software will enable the analyses of the assessment of phenotypic markers that are expressed on immune cells. The samples will also be isolated for specific immune cell including B and T-cells, which will be stored in a secure -80°C freezer. Plasma will also be collected from these samples to assess circulating factors, to be assessed by commercially-available kits.

Human valve samples will be formalin fixed and paraffin embedded before being shipped to a third party provider (Azenta life sciences) for spatial RNA sequencing at a later date.

Sample storage and transfer

Blood and valve samples will be taken by Dr Harding (or a dedicated member of the research team) and will be transferred to the laboratory using secure sample transfer boxes. All blood and heart valve tissue samples will be processed within one hour of collection. Blood samples will be stored within a secure -80C freezer at the Heart Centre, where the Centre for Biochemical Pharmacology is based. All samples will be fully anonymized.

16. Safety reporting

The study involves taking blood samples from the participant. One of the main risks of blood tests are discomfort and bruising at the site where the needle goes in. To minimise bruising, the participant will be encouraged to put pressure at the puncture site immediately after the sample has been taken.

17. Monitoring and auditing

The Sponsor or delegate retains the right to audit any study, study site or central facility. In addition, any part of the study may be audited by the funders where applicable.

On-site monitoring will be performed as per the study monitoring plan. Monitoring will include source data verification.
Monitoring of the centre may be undertaken by a trained study monitor or monitors, to verify the study documentation. The sponsor reserves the right to audit trial sites, subject to giving a suitable period of notice (minimum period of notice 10 working days).

18. Study committees

A study management group will be in place where we will hold monthly meetings. The study management group will comprise of the investigators directly involved in this study:

Dr Daniel Harding (CI)
Professor Simon Woldman, Consultant Cardiologist
Professor Federica Marelli-Berg, British Heart Foundation Chair of Cardiovascular Immunology (QMUL)

19. Finance and funding

This study is in the process of peer review and a funding application has been made to the Barts Charity (ID 27360).

20. Insurance and indemnity

The insurance that Queen Mary has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

21. Dissemination of research findings

In addition to publishing our key findings in peer-reviewed articles, we plan to disseminate aspects of our research via newsletters, web based information, and open forum patient information days. The proposed research will be incorporated into our current public engagement schemes

22. References

1. Harding D, Prendergast B, Kutarski A, Rouzet F, Bernard BC. Advanced imaging improves the diagnosis of infective endocarditis. *F1000Research 2018 7:674*. 2018;7:674. doi:10.12688/f1000research.13791.1

2. Cresti A, Chiavarelli M, Scalese M, et al. Epidemiological and mortality trends in infective endocarditis, a 17-year population-based prospective study. *Cardiovasc Diagn Ther*. 2017;7(1):27-35. doi:10.21037/CDT.2016.08.09

3. Harding D, Cahill TJ, Redwood SR, Prendergast BD. Infective endocarditis complicating transcatheter aortic valve implantation. *Heart*. 2020;106(7). doi:10.1136/heartjnl-2019-315338

4. Holland TL, Baddour LM, Bayer AS, Hoen B, Miro JM, Fowler VG. Infective endocarditis. *Nat Rev Dis Primers*. 2016;2(1):16059. doi:10.1038/nrdp.2016.59

5. Garnier JL, Touraine JL, Colon S. Immunology of infective endocarditis. *Eur Heart J*. 1984;5(suppl_C):3-9. doi:10.1093/EURHEARTJ/5.SUPPL_C.3

6. Fanti S, Stephenson E, Rocha-Vieira E, et al. Circulating c-Met-Expressing Memory T Cells Define Cardiac Autoimmunity. *Circulation*. 2022;146(25):1930-1945. doi:10.1161/CIRCULATIONAHA.121.055610

7. Investigating infective endocarditis: teaching hospitals to choose wisely. *British Journal of Cardiology*. Published online 2023. doi:10.5837/BJC.2023.032

This protocol is based on JRMO Protocol template for Research Studies; v4.0 07.04.2022