The *InflammaTENSION* Study

A study of the roles of the immune and inflammatory systems in hypertension.

Chief Investigators:

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This study will be performed according to the Research Governance Framework for Health and Community Care (Second edition, 2006) and WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects 1964 (as amended).

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Protocol Approval

The InflammaTENSION **Study**

A study of the roles of the immune and inflammatory systems in hypertension.

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Abbreviations

ABPM	Ambulatory Blood Pressure Monitoring
CIMT	Carotid intima-media thickness
CRF	Glasgow Clinical Research Facility
BHF	British Heart Foundation
DSS	Digital Symbol Substitution
EC	Electronic Cigarettes
EQ-5D	European Quality of Life-5 Dimensions
FMD	Flow Mediated Dilation
GM/HC	General Medicine/Hypertension Clinic (Queen Elizabeth University Hospital)
GWAS	Genome wide association study
GP	General Practice
HTN	Hypertension
IDQ	the Interheart Diet Questionnaire
IPAQ	the International Physical Activity Questionnaire
InflammaTENSION	A study of the roles of the immune and inflammatory systems in hypertension.
MCQ	the Mild Cognitive Impairment Questionnaire
MoCA	Montreal Cognitive Assessment
NHSGGC	NHS Greater Glasgow and Clyde
PFTs	Pulmonary Function Tests
PIL	Participant information leaflet
QEUH	Queen Elizabeth University Hospital
RCT	Randomised Controlled Trial

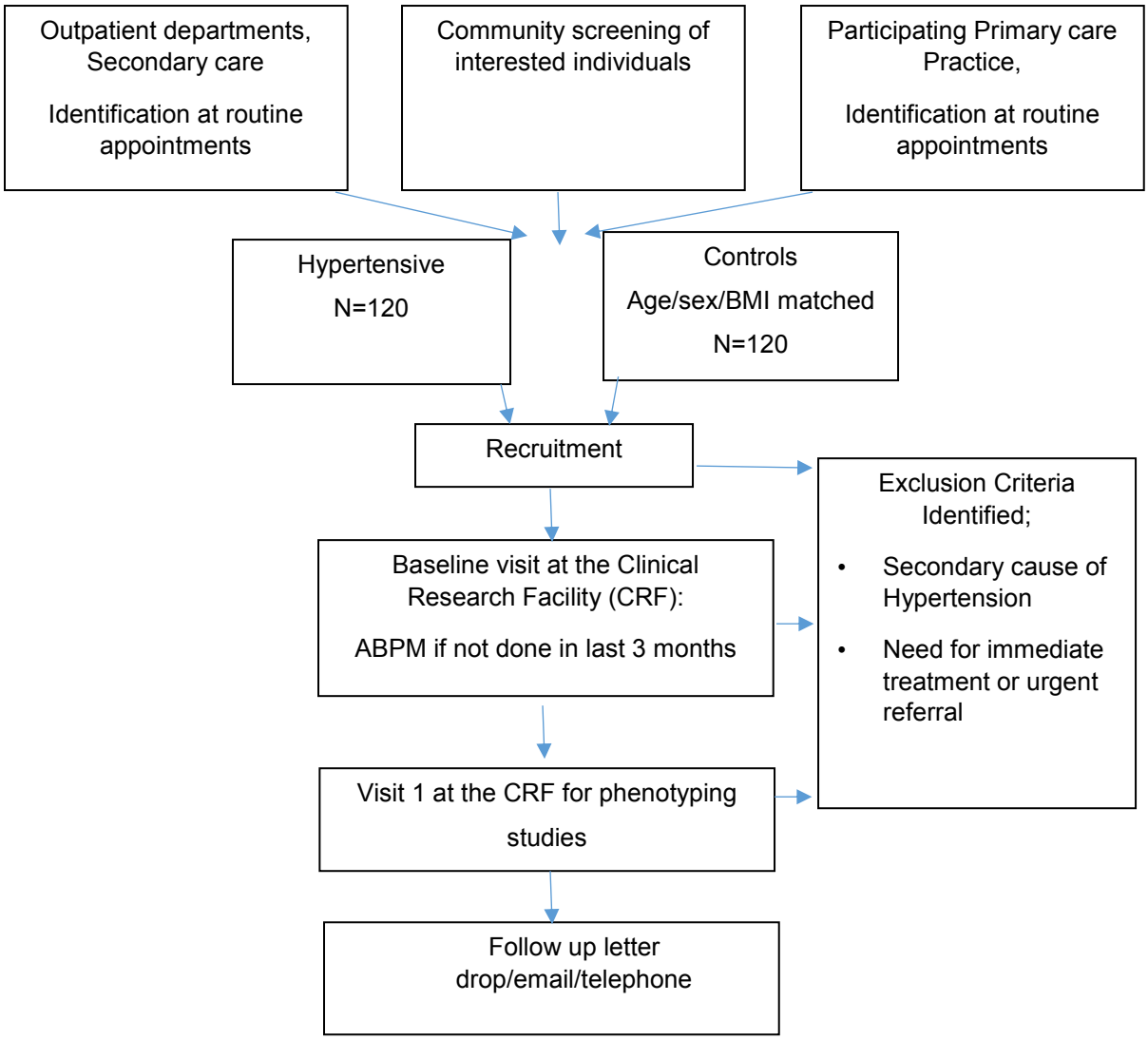
Study Synopsis

Title of Study:	A study of the roles of the immune and **inflamma**tory systems in hyper**tension**. (InflammaTENSION Study).
Study Centre:	Institute of Cardiovascular and Medical Sciences
Duration of Study:	36 months
Primary Objective:	To define the cytokine and cellular immune signature of primary hypertension.
Methodology:	Cross sectional clinical/laboratory study.
Secondary Objectives:	• To define the relationships and predictive value of the immune signature of hypertension and clinical phenotypes of hypertension ➢ Predictive value of immune signature for blood pressure parameters measured by ambulatory blood pressure measurements (ABPM) ➢ Predictive value of immune signature for endothelial function assessed by Endo-PAT2000 and flow mediated dilatation (FMD) both complementary non-invasive techniques. ➢ Predictive value of immune signature for vascular stiffness and central pressure assessed by SphygmoCor ➢ Predictive value of immune signature for renal function parameters ➢ Predictive value of immune signature for cognitive function • To define genetic determinants of immune signature of hypertension.
Primary Endpoint:	Cytokine and cellular immune signature of primary hypertension evaluated by association between hypertensive status (assessed by ABPM) and immune signature (analysed as a whole as well as individual pro- and anti- inflammatory cytokine levels and discreet immune cell populations).
Rationale:	Experimental data show the presence of immune and inflammatory systems dysregulation in hypertension. Understanding of the inflammatory and immune nature of hypertension is currently based on studies in rodent models of hypertension, but is supported by human epidemiological and genome wide association studies (GWAS) studies. It is now essential to identify key checkpoints and inflammatory mechanism(s) involved in human hypertension in comprehensive and sufficiently powered studies, which will then be able to guide subsequent in-depth hypothesis-driven mechanistic studies. This approach may provide the basis for future randomized clinical trials (RCTs).
Methodology:	Cross sectional clinical/laboratory study of 120 primary hypertensive and 120 age, sex and BMI matched - controls Cardio renal studies/investigations as well as cognitive dysfunction assessment will be performed during 1 or 2 visits depending on recent ABPM availability and patient preference
Sample Size:	120 hypertensive 120 controls

Screening:	Initial Screening by the clinical care team at a routine outpatient appointment with subsequent verification by a member of InflammaTENSION team (who are also member of the clinical care team for hypertensive patients).
Registration/Randomisation:	No randomization
Main Inclusion Criteria:	Age between 18-50 years Cases: Office blood pressure ≥140 and ≥90 Controls: Office blood pressure <140 and <90 and age, sex and BMI matching to cases
Main Exclusion Criteria:	**(a)** Age >50 years old; (b) Secondary hypertension (including e.g. adrenal tumours, pheochromocytoma, renal artery stenosis; thyroid disease) **(c)** Acute inflammatory disorders incl. flu, rhinitis, sinusitis etc. within 3 weeks; hospitalization within the past 3 months; Life expectancy of < 3 years; History of alcohol/substance abuse **(d)** Allergic disorders; chronic infections, COPD, tuberculosis; hepatitis B or C; pneumonitis, bronchiectasis; pericardial or pleural effusion, ascites; liver disease; **(e)** Chronic inflammatory/autoimmune conditions such (e.g. SLE, rheumatoid arthritis, ulcerative colitis/Crohn's disease; non-basal cell malignancy or myelo- or lymphoproliferative disease within the past 5 years; known HIV+; Immunizations (3 months); pulmonary hypertension; **(f)** Pregnancy, nursing; **(g)** History of symptomatic coronary artery disease (events) or heart failure; **(h)** BMI>35, diabetes/glucose intolerance (fasting glucose, HbA1; testing, glucose challenge where indicated); **(i)** Known albuminuria/microalbuminuria; GFR<60mL/min/1.73m2. **(j)** Any chronic concurrent treatment. Use of systemic or local steroids/immunosuppressive agents (within 6 months) of the inclusion; Current (within past 3 months) use of anti-hypertensive medication; (k) Major depressive illness or other psychiatric conditions. (l) Participants who decline participation in the study or who are unable to provide informed consent
Product, Dose, Modes of Administration:	N/A
Duration of Treatment:	N/A
Statistical Analysis:	Institute of Cardiovascular and Medical Sciences; University of Glasgow (Dr John McClure)

InflammaTENSION Study Flowchart

Figure 1: InflammaTENSION Study Flowchart



Study Schedule of InflammaTENSION Study

Table 1: Study Schedule of InflammaTENSION Study

Study Schedule	**Procedure Details**	
Recruitment	Clinical care team at a routine outpatient appointment / Letter Drop/ traditional and social media/ SHARE registry/ word of mouth/ referral from colleagues	
Registration	Contact InflammaTENSION investigator; if ABPM is available within past 3 months - patient will be able to choose preference for combined or separate Baseline visit and Visit 1.	
Baseline Visit at CRF (Visit 0)	Explanation of InflammaTENSION Study Answering of Participant Questions Detailed Inclusion and Exclusion Criteria Consent	
	Study Procedures (Please refer to The InflammaTENSION Study: Manual of Procedures for more details regarding the study procedures)	Demographic History Medical History Height and Weight Urine Sample Heart Rate, Office Blood Pressure measurement – 3 times following 5 minutes of rest Venous Blood Sample (40ml) for Blood Tests and Immune signature/Biomarker analysis Venous Blood Sample (10ml) for storage for subsequent genetic analysis Ambulatory Blood Pressure Monitor Installation if no ABPM performed within past 3 months Subjects will be given an IDQ questionnaire (The Interheart Diet Questionnaire) to be returned during visit 1

Booking of Visit Appointments	Following Baseline Visit, visit 1 appointment will be booked if not planned together with Baseline Visit. The InflammaTENSION investigator will liaise with each participant to arrange an appointment times,	Booking of Visit 1 (0-72 hours post Baseline Visit)	Establishing preferred contact route (email/letter) for follow up letter drop and Final Telephone Visit (3.5 months +/- 14 days following Visit 1)
Visit 1 0-72h post Baseline Visit	Study Procedures (Please refer to The InflammaTENSION Study: Manual of Procedures for more details regarding the study procedures)	This visit will take approximately 2-3.5 The following study procedures will be performed at the final visit. Questionnaires ➢ MCQ The Mild Cognitive Impairment Questionnaire ➢ MoCA Montreal Cognitive Assessment ➢ DSS Digital Symbol Substitution ➢ IPAQ The International Physical Activity Questionnaire ABPM monitor will be returned Carotid Intima-Media Thickness Flow Mediated Dilation of Brachial Artery Endo-PAT2000 Sphygmocor study	
Letter Drop/ Telephone Visit (3.5 months +/- 14 days from Baseline Visit)	Study Procedures (Please refer to The InflammaTENSION Study: Manual of Procedures for more details regarding the study procedures)	• Letter sent containing questionnaire regarding changes in medical history since Visit 1 and offering telephone contact from InflammaTENSION investigator. • Obtaining information on participant health status changes; changes in demographics; changes in medical history • Verification if any cause for secondary hypertension has been identified.	

1. Introduction

The *InflammaTENSION* Study

A study of the roles of the immune and inflammatory systems in hypertension.

1.1 Background

The overarching theme of the proposal Hypertension is a common disease impacting 1 billion people worldwide, which leads to catastrophic cardiovascular complications, including heart failure, dementia, myocardial infarction and stroke - all of which carry a severe socioeconomic burden. In spite of many years of research, the cause of primary hypertension remains unknown and this disease is uncontrolled in a large proportion of patients. By interrogating the key hypothesis that inflammatory dysregulation fundamentally controls development of hypertension and vascular remodelling, **InflammaTENSION** provides a new paradigm for the management of the disease, with the potential to lead to the identification of novel therapeutic targets to control blood pressure and limit target organ damage. InflammaTENSION will result in the discovery of novel biomarkers, which may identify patients who could benefit from such immune targeted therapies. Importantly, we already made the seminal observation that the immune system not only mediates target organ damage, but defines the roles of pro-inflammatory T cells, monocytes, as well as anti-inflammatory T regulatory cells in the disease process[1-4]. However, our current knowledge remains very fragmented and so far has not been applied to human pathology. InflammaTENSION will for the first time advance the knowledge procured in rodent models into human studies. By combining clinical translational and model mechanistic studies it will identify novel inflammatory factors that can control immune mechanisms of hypertension. In detail, over the course of the programme we will: (1) characterize the immunophenotypic signature of human hypertension; (2) define key concepts in cytokine biology of hypertension and (3) understand how chronic cytokines implicated in hypertension regulate the T cell dependent mechanisms of hypertension. InflammaTENSION will also go beyond current state-of-the-art diagnostic methods,with comprehensive combination of immunology and cardiovascular disease to create a new understanding of how the immune system may lead to human hypertension and vascular remodelling. Such a coordinated and integrative programme to better understand the role of dysregulation of the immune system in human hypertension will have major impact on the field, enabling translation of these exciting findings to clinical practice.

Unmet need to be investigated: There is an urgent need for a better understanding of the mechanisms of hypertension (HTN), as it remains a major cause of death and disability in Europe and worldwide and the prediction is that its prevalence will increase by 60% over the next 25 years. The disease affects 30% of adults, with an additional 30% considered at high risk of hypertension[1]. Its prevalence increases with age with 70% of adults older than 70 being afflicted with this disease. Thus within the ageing society, the socioeconomic consequences are particularly marked[5], with many elderly patients developing severe cardiovascular complications such as heart failure, stroke, myocardial infarction, vascular dementia and renal failure. Drug therapy for hypertension improved dramatically between 1975 and 1985, with the addition of angiotensin receptor antagonists, angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers. Since the mid-1980s, however, no new classes of drugs have been successfully introduced to treat hypertension. This is unfortunate, because up to 40% continue to have elevated blood pressure despite the use of multiple antihypertensive agents. While partially related to poor treatment compliance, this highlights

our insufficient understanding of the underlying mechanisms of this disease. While some cases of hypertension are due to single gene mutations[6], or underlying correctable causes such as renal artery stenosis, pheochromocytoma or adrenal adenoma[7], these are uncommon and the cause of the majority of cases of adult hypertension are unknown. In these cases, neurohumoral factors such as angiotensin II play key roles, as drugs interfering with this pathway are anti-hypertensive. Systemic vascular resistance is generally elevated in hypertension[8-10], and vasodilators lower blood pressure, which would suggest that hypertension, is a *vascular disease*. In contrast, the genetic disorders causing hypertension often affect sodium transport in the distal nephron[6]. Therefore we would like to assess genetic difference in cytokine and chemokines in the study cases and controls. Transplant of kidneys lacking the angiotensin II AT1a receptor into wild-type mice causes resistance to angiotensin II-induced hypertension[11] and diuretics are effective anti-hypertensive agents[12], which in turn suggests that *the kidney* is a major cause of high blood pressure. Finally, there is ample evidence that the *central nervous system*, and in particular the circumventricular organs surrounding the third ventricle, play a critical role in hypertension[13]. These seemingly disparate roles of the vasculature, the kidney and the CNS make the aetiology of hypertension very difficult to comprehend. Importantly, we have made an observation that helps link the vasculature, the kidney and the CNS in the genesis of hypertension, as all appear to be related to a common inflammatory mechanism. These studies have initiated a new research area, which over the past few years has led to the better understanding of inflammation in hypertension.

While the evidence for the role of immune mechanisms in hypertension has been obtained primarily in rodent models, epidemiological and genetic evidence strongly supports this. Blood pressure increases with the quartile of C-reactive protein[14],[15] and cytokine levels such as TNF-α and IL-6 are consistently increased in hypertension[16-18], and may convey risk of developing the disease[16]. Hypertension is highly prevalent in immune mediated diseases such as rheumatoid arthritis or psoriasis[19], [20]. A recent small study has shown an increase of senescent CD8+T cells (CD28null) in peripheral blood and target organs in human hypertension[21]. The T cell modulating agent, mycophenolate mofetil, as well as anti-TNF-α treatments lower blood pressure not only in rodents[22],[23], but also in humans[24], suggesting that immune targeted interventions may pose a feasible future approach, if we are able to identify its mechanisms. Importantly, both GWAS studies[25] and gene expression signatures of hypertension[26] strongly point towards the role of the immune system and inflammation. SH2B3/LNK gene encoding T cell activation modulator is a top key driver of hypertension in recent systems biology analysis[25].

1.2 Rationale

Understanding of the inflammatory and immune nature of hypertension is currently based on studies in rodent models of hypertension, but is supported by human epidemiological and GWAS studies. It is now essential to identify key checkpoints and mechanisms of inflammatory mechanism(s) of human hypertension in comprehensive and sufficiently powered studies, which will then be able to guide subsequent in-depth hypothesis-driven mechanistic studies. This approach may provide the basis for future randomized clinical trials (RCTs).

By interrogating the key hypothesis that inflammatory dysregulation fundamentally controls development of hypertension and vascular remodelling, **InflammaTENSION** provides a new paradigm for the management of the disease, with the potential to lead to the identification of novel therapeutic targets to control blood pressure

and limit target organ damage.

We hypothesize that primary hypertension is associated with distinct pattern of changes within immune/inflammatory systems consisting of cytokines/chemokines and individual immune cell populations.

The data generated from the InflammaTENSION study will be invaluable for identification of novel biomarkers and therapeutic targets in hypertension.

Biomarker analysis will be carried out using standard laboratory techniques for cytokines and chemokines as follows;

Luminex (Invitrogen Cytokine/Chemokine/Growth Factor 45-Plex Human ProcartaPlex™ Panel 1
Target List: BDNF; Eotaxin/CCL11; EGF; FGF-2; GM-
CSF; GRO alpha/CXCL1; HGF; NGF beta; LIF; IFN alpha; IFN gamma; IL-1 beta; IL-1 alpha; IL-1RA; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8/CXCL8; IL-9; IL-10; IL-12 p70; IL-13; IL-15; IL-17A; IL-18; IL-21; IL-22; IL-23; IL-27; IL-31; IP-10/CXCL10; MCP-1/CCL2; MIP-1 alpha/CCL3; MIP-1 beta/CCL4; RANTES/CCL5; SDF-
1 alpha/CXCL12; TNF alpha; TNF beta/LTA; PDGF-BB; PLGF; SCF; VEGF-A; VEGF-D

O-Link:Proseek® Multiplex Inflammation I96×96 is a high-
throughput, multiplex immunoassay enabling analysis of 92 inflammation-
related protein biomarkers across 96 samples simultaneously. This high level of multiplexing is achieved witho ut any compromise on data quality, thanks to our proprietary Proximity Extension Assay (PEA) technology

If the data generated from such a trial demonstrate that hypertension has a very distinct immune signature – it can be used to stratify patients for future therapies and may provide proof-of-concept for immune targeted therapies thus leading to the development of long term studies.

1.3 Prior Experience of Intervention in Cardiovascular Disease

In a small pilot study published in July 2016 we were able to identify that hypertension is associated with key changes of memory T cells and with changes of immune factors (IL-17, IFN-g)[27]. This justifies performing a comprehensive investigation to identify detailed immune signature of hypertension.

1.4 Study hypothesis

We hypothesize that primary hypertension is associated with distinct pattern of changes within immune/inflammatory systems consisting of cytokines/chemokines and individual immune cell populations and that this immune signature corresponds with vascular, renal or cognitive phenotypes of hypertension.

2. Study Objectives

The InflammaTENSION pilot study is an observational study aiming to define the cytokine and cellular immune signature of primary hypertension.

- **Primary Endpoint**

  Cytokine and cellular immune signature of primary hypertension

- **Secondary Endpoints**

1. Relationships and predictive value of the immune signature of hypertension and clinical phenotypes of hypertension
   - Predictive value of immune signature for blood pressure parameters measured by ambulatory blood pressure measurements (ABPM)
   - Predictive value of immune signature for endothelial function assessed by Endo-PAT2000 and flow mediated dilatation (FMD) both complementary non-invasive techniques.
   - Predictive value of immune signature for vascular stiffness and central pressure assessed by Sphygmocor
   - Predictive value of immune signature for renal function parameters
   - Predictive value of immune signature for cognitive function
2. To define genetic determinants of immune signature of hypertension which could be used for future mendelian randomization studies.

3. Study Design

The InflammaTENSION study will be performed according to the Research Governance Framework for Health and Community Care (Second edition, 2006).

3.1 Study Population

120 hypertensive subjects and 120 controls will be recruited into the trial (both male and female).

3.2 Inclusion criteria

- Age between 18-50 years
- Cases: Office blood pressure ≥140 and ≥90.
  Controls: Office blood pressure <140 and <90 and age, sex and BMI patching to cases

3.3 Exclusion criteria

(a) Age >50 years old;

(b) Secondary hypertension (including e.g. adrenal tumours, pheochromocytoma, renal artery stenosis; thyroid disease)

(c) Acute inflammatory disorders incl. flu, rhinitis, sinusitis etc. within 3 weeks; hospitalization with an inflammatory condition within the past 3 months; Life expectancy of < 3 years; History of alcohol/substance abuse

(d) Allergic disorders; chronic infections, COPD, tuberculosis; hepatitis B or C; pneumonitis, bronchiectasis; pericardial or pleural effusion, ascites; liver disease;

(e) Chronic inflammatory/autoimmune conditions such (e.g. SLE, rheumatoid arthritis, ulcerative colitis/Crohn's disease; non-basal cell malignancy or myelo- or lymphoproliferative disease within the past 5 years; known HIV+; Immunizations (3 months); pulmonary hypertension;

(f) Pregnancy, nursing;

(g) History of symptomatic coronary artery disease (events) or heart failure;

(h) BMI>35, diabetes/glucose intolerance (fasting glucose, HbA1; testing, glucose challenge where indicated);

(i) Known albuminuria/microalbuminuria; GFR<60mL/min/1.73m2.

(j) Any chronic concurrent treatment. Use of systemic or local steroids/immunosuppressive agents (within 6 months) of the inclusion; current (within past 3 months) use of anti-hypertensive medication;

(k) Major depressive illness or other psychiatric conditions.

(l) Participants who decline participation in the study or who are unable to provide informed consent

3.4 Identification of Participants and Consent

3.4.1. Participant Identification

Participants will be identified through:

- Potential participants will be identified by members of the direct clinical hypertension care team at *QEUH Glasgow Blood Pressure Clinic* (where PI – Prof Guzik and Prof Delles are part of the team), supported by the research nurse team

- Other secondary care routine out-patient appointments where potential participants and controls can be identified and invited to participate.
- Participants may also be identified though SHARE, primary and secondary care data bases and direct identification of potential participants through the SPCRN/GGC primary care team.
- Potential participants for the age/sex/BMI matched control group will be identified by a member of the direct clinical pre-OP orthopaedics care team (from among subjects with planned minor surgeries (e.g. arthroscopy), supported by the research nurse team
- Potential participants may also be identified through traditional and social media including newspaper advertisement, press release, posters in NHS and public sites as well as word of mouth and referral from colleagues.

Potential participants will be approached in one of two ways: 1) approach in person by the clinical care team at a routine outpatient appointment. Those who demonstrate interest will be given the patient information leaflet (PIL) and asked for verbal consent for their details to be passed to the research team. The PIL will contain a contact email address and telephone number to allow patients to "opt in" to the study or get in contact for further information. If there has been no contact from a patient 48 hours after being given the PIL, the patient will be telephoned by the clinical fellow or research nurse to ascertain interest in participation in the study; 2) letter drop: letters posted to the patient by the principal investigator with information about the study. The same contact information will be provided for patients contacted by letter drop to allow them to opt in to the study. These patients will also be contacted 5-7 days after posting the letter by the clinical fellow to assess interest in participating in the study.

Interested participants will be able to register their interest by contacting the InflammaTENSION investigators by:

- Email
- Telephone
- Posting the attached reply slip in the prepaid envelope

Both the poster and letters will contain contact registration details:

- Study specific email address:gg-uhb.inflammatension@nhs.net
- InflammaTENSION investigator study specific mobile number:
- InflammaTENSION investigator telephone number: 0141 330 7590

3.4.2. Participant Registration

The telephone registration process will include:

- Obtaining participants contact details: Name, Date of Birth, Address, Telephone Number and Email
- Explanation of the study
- Initial Eligibility Screen (with particular focus on verification of lack of known exclusion criteria)
    - Ineligible participants will be asked if they would like a referral to *Glasgow Blood Pressure Clinic, QEUH*.
- Eligible participants will be invited for a baseline visit, and an appointment date and time will be made, a participant information leaflet (PIL) will be sent via post or email; depending on the participants' preference.

3.4.3. Consent at Baseline Visit

All participants will have to provide written consent to take part in the study, the consent taken will be inclusive for the InflammaTENSION study and must be obtained before any of the study procedures can commence. All participants will be provided with a signed copy of their consent form. .

The Chief Investigator (PI) will retain overall responsibility for the informed consent of participants and will ensure that any person delegated responsibility to participate in the informed consent process is duly authorized, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki.

Informed consent will be obtained prior to the participant undergoing procedures that are specifically for the purposes of the study and are out-with standard routine care.

The right of a participant to refuse participation without giving reasons will be respected.

Before consent is taken the InflammaTENSION investigator will:

- Ensure that the participant has read the PIL and has had ≥ 24 hours to reflect upon the information
- Explain the study to the participant
- Go through detailed inclusion and exclusion criteria for the study**
- Answer any of their questions relating to the study
- Explain that participation in the study is entirely voluntary and they do not have any obligation to take part in the study and they can leave the study at any point***

The process of consent will involve:

- a discussion between the potential participant and an individual knowledgeable about the research about the nature and objectives of the trial and possible risks associated with their participation
- the presentation of written material (e.g., information leaflet and consent document which must be approved by the REC and be in compliance with GCP, local regulatory requirements and legal requirements)
- the opportunity for potential participants to ask questions
- Assessment of capacity: for consent to be ethical and valid in law, participants must be capable of giving consent for themselves. A capable person will:
    - understand the purpose and nature of the research
    - understand what the research involves, its benefits (or lack of benefits), risks and burdens
    - understand the alternatives to taking part
    - be able to retain the information long enough to make an effective decision
    - be able to make a free choice
    - be capable of making this particular decision at the time it needs to be made (although their capacity may fluctuate, and they may be capable of making some decisions but not others depending on their complexity)
    - where participants are capable of consenting for themselves but are particularly susceptible to coercion, it is important to explain how their interests will be protected

A person is assumed to have the mental capacity to make a decision unless it is shown to be absent. Mental capacity is considered to be lacking if, in a specific circumstance, a person is unable to make a decision for him or herself because of impairment or a disturbance in the functioning of their mind or brain. In practice for participants with mental

incapacity this means that they should not be included in clinical trials if the same results can be obtained using persons capable of giving consent and should only be included where there are grounds for expecting that their taking part will be of direct benefit to that participant, thereby outweighing the risks.

3.5 Withdrawal of Subjects

The participant will remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment and will be provided with a contact point where he/she may obtain further information about the study and where they can receive further needed care. Patient's withdrawn and needing clinical care will be referred for a visit to *Glasgow Blood Pressure Clinic at QEUH*. Referrals to *Glasgow Blood Pressure Clinic* will be a written letter and participants will be directed to NHSGGC Prof Guzik/Prof Delles led clinic.

Where a participant is required to re-consent or new information is required to be provided to a participant, the PI will ensure this is done in a timely manner.

The investigator can withdraw participants from the study in the event of inter-current illness or identification of a secondary cause for hypertension. Participants who withdraw from the study will be replaced with additional participants until 120 participants/arm have attended the final study visit.

***Verbal consent will be obtained from the participants prior to eligibility screening and/or referral to NHSGGC to QEUH General Medicine/Hypertension Clinic*

****Participants who are either ineligible or no longer wish to take part in the study will be asked if they would like a referral to QEUH General Medicine/Hypertension Clinic for further diagnosis and treatment, and they will be provided with the contact details for QEUH General Medicine/Hypertension Clinic. Referrals to QEUH Glasgow Hypertension Clinic will be a written letter and participants will be directed to NHSGGC Prof Guzik/Prof Delles led clinic.*

4. Study Schedule and Trial procedures

NB:

- ***Please refer to the InflammaTENSION Manual of Study Operations for an in-depth detail of the study procedures***
- ***Please refer to Table 1: Study Schedule***

4.1 Recruitment

Once identified and consented as described in section 3.4 the study visit activities can commence.

4.3 Study Visit Information

- **Location:** All study visits will take place at Glasgow Clinical Research Facility 5th Floor, Neurosciences Building Queen Elizabeth University Hospital Campus 1345 Govan Road Glasgow G51 4TF
- Number of Study Visits: All participants will attend a baseline study visit and visit 1 -either separately or combined depending on the participants wishes and availability of ABPM within past 3 months.
- An additional telephone contact and verification will be performed 3.5months +/- 2 weeks after final study visit to ensure secondary causes of hypertension would have not been identified in subsequent months.
    - In case of clinical need, and if subjects are agreeable, they will be referred to QEUH General Medicine/Hypertension Clinic, led by NHSGGC Prof Guzik/Prof Delles for further diagnosis and treatment..

4.4 Study Schedule and Trial Procedures

Table 1: Schedule of InflammaTENSION Study procedures

Study Procedure	Baseline Visit 0 at CRF	Visit 1 at CRF	Telephone visit/Letter Drop
Timeframe	V0	V0+(0-72h)	V1+12m±2w
Demographic History	X	-	x
Medical History	X	-	x
Height and Weight	X	-	x
Urine Sample	X	-	-
Heart Rate	X	-	-
Office Blood Pressure measurement – 3 times following 15 minutes of rest	X	-	-
Venous Blood Sample (40ml) for Blood Tests and Immune signature/Biomarker analysis	X	-	-
Venous Blood Sample (10ml) for storage for subsequent genetic analysis	X	-	-
Ambulatory Blood Pressure Monitor Installation if no ABPM performed within past 3 months	X	-	-
MCQ	-	X	-
MoCA	-	X	-
DSS	-	X	-
IDQ	-	X	-
IPAQ	-	X	-
ABPM monitor will be returned	-	X	-
Carotid Intima-Media Thickness	-	X	-
Flow Mediated Dilation of Brachial Artery	-	X	-
Endo-PAT2000	-	X	-
Sphygmocor study	-	X	-

Key	- Not performed	X Performed

h hours w – weeks m - months

General Considerations

In hypertensive subjects' study visit(s) will be arranged as soon as possible after initial diagnosis of hypertension and before initiation of the treatment for hypertension. The study visit will not delay initiation of therapy in any way and will be fitted in between the hypertension work-up and subsequent clinic visit. In subjects recruited from GHBPC (QEUH) this will be organized following initial diagnostic ABPM which is routinely performed prior to patient being seen by a consultant. In patients recruited from elsewhere – this will be arranged as soon as possible after identification and GP will be informed about ABPM which will be performed as part of the study by a letter from InflammaTENSION clinical team member.

Secondary hypertension will be excluded as per routine clinical practice. We also planned a letter drop questionnaire or telephone visit/data review 3-4 months after initial visit in order to verify secondary/primary status of hypertension. While we have planned baseline visit and Visit 1 separately with ABPM being performed in between, subjects who do not require ABPM (it is available within past 3 months) we will offer an option to combine these visits in one day for the patients.

Baseline Visit (Visit 0)

Meeting: The InflammaTENSION investigators will meet the participants at reception of the CRF.

Estimated Duration of Baseline Visit: Approximately 0.5 - 1 hours

Recording of Study Information: The data from the study procedures will be recorded in the electronic case report form (e.g. CASTOR EDC).

Study Procedures Performed at the Baseline Visit will include:

(Please refer to the InflammaTENSION Study Manual of Procedures for more details relating to the study procedures)

- Demographic History
- Medical History
- Height and Weight
- Urine Sample
- Office Blood Pressure measurement – 3 times following 5 minutes of rest
- Venous Blood Sample (40ml) for Blood Tests and Immune signature/Biomarker analysis
- Venous Blood Sample (10ml) for storage for subsequent genetic analysis
- Ambulatory Blood Pressure Monitor Installation if no ABPM performed within past 3 months
- Subjects will be given an IDQ questionnaire (The Interheart Diet Questionnaire) to be returned during visit 1

All procedures will be performed in both Cases and Control groups including ABPM as it will ensure that hypertension is not missed by office blood pressure readings.

Anonymisation

- Patient data will be pseudo-anonymised with a study number given to the patient. This study number will be used for blood tubes. The study specific number will be used for all storage of electronic and paper data relating to the;
    - Vascular function determinations
    - Immune signature determinations (cytokine/chemokine and cellular)
    - Cognitive function questionnaires
    - Will be blinded towards hypertension status and group allocation.
- Only the Chief Investigator, and designated and trained research fellow, who is a member of the direct care team, will have access to the participants' personal data during the study. These procedures will comply with the 1998 Data Protection Act. Biological samples collected from participants as part of this study will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities will meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

Visit 1 (CRF)

Study Procedures performed at Visit 1 at CRF (either in combination with baseline or up to 72h after baseline Visit)

NB: There is no need for a study specific ABPM if a routine care ABPM has been performed within the past 3 months.

This visit will take approximately 2-3.5 the following study procedures will be performed at the final visit.

- Questionnaires
  - ✓ MCQ The Mild Cognitive Impairment Questionnaire
  - ✓ MoCA Montreal Cognitive Assessment
  - ✓ DSS Digital Symbol Substitution
  - ✓ IDQ The Interheart Diet Questionnaire
  - ✓ IPAQ The International Physical Activity Questionnaire
- ABPM monitor will be returned
- Carotid Intima-Media Thickness
- Flow Mediated Dilation of Brachial Artery
- Endo-PAT2000
- Sphygmocor study

Participants will be thanked for taking part in the InflammaTENSION study.

Letter drop / Telephone contact (3.5 months +/- 14days)

A follow - up letter will be sent by post or e-mail to all participants asking for filling in a questionnaire regarding the key changes in medical status since Visit 0. It will also announce possible telephone contact if no response is received to initial letter

The telephone registration process will include:

- Obtaining information on participant health status changes; changes in demographics; changes in medical history and in particular verification if any cause for secondary hypertension has been identified.

4.5 Study Outcome Measures

4.5.1 Primary Outcome Measure

Cytokine and cellular immune signature of primary hypertension.

Association between hypertensive status assessed by ABPM and immune signature analysed as a whole as well as individual pro- and anti- inflammatory cytokine levels and discreet immune cell populations.

4.5.2 Secondary Outcome Measure

- Association of selected cytokine/chemokine levels with vascular phenotypes
    - endothelial function assessed by Endo-PAT2000, a non-invasive technique.
    - Large vessel endothelial function assessed by brachial artery flow mediated dilatation, a non-invasive technique.
    - Changes in cardiovascular parameters through non-invasive hemodynamic measurements
- Association of selected cytokine/chemokine levels with renal phenotypes
    - GFR; renin levels; aldosterone levels
- Association of selected cytokine/.chemokine levels with cognitive dysfunction
    - Results of MCQ, MoCA, DDS
- Association of selected cytokine/.chemokine levels with genetic variation assessed by GWAS

4.6. Incidental findings

Any incidental findings observed during the research procedures will be acted upon: this means that the PI takes responsibility to notify the patients GP and refer on for specialist follow up as appropriate and with the patient or participants permission.

Patients who would benefit from hypertension/cardiovascular risk factor management will be referred to and provided with contact details for NHSGGC Prof Guzik/Prof Delles led clinic *at the QEUH General Medicine/Hypertension Clinic for further diagnosis and treatment.*

6. Statistics and Data Analysis Plan

6.1 Statistical Analysis Plan

The InflammaTENSION study will have a comprehensive Statistical Analysis Plan, which will govern all statistical aspects of the study, and will be authored by the Study Statistician (Dr John McLure). To gain additional insight, variable selection analysis will be used to identify the best predictors of hypertension and its vascular outcomes (endothelial function/vascular compliance). To do this we will draw the expertise of local bioinformaticians (Dr John McClure) and of Professor Andrew Yates, a theoretical immunologist at University of Glasgow with experience in the modeling of cytokine networks and in machine learning approaches to TCR repertoire analysis. We anticipate using exploratory unsupervised approaches such as Principle Component Analysis and clustering may be used on the cytokine profiles to identify signature patterns of cytokine expression that may allow a reduction of dimensionality. One can also associate each patient's cytokine profile with a score - either one dimensional measure of disease severity or perhaps multi-factorial measures, combining severity with demographic data, for example, and use supervised learning approaches such as support vector machines or neural networks to generate classifiers or predictors of outcomes.

6.2 Sample Size

The sample size will comprise of 120 participants per study arm (120 cases + 120 controls).
Based on preliminary data: at least 116 subjects will allow us to detect an IL-6 difference of 25% at a two-sided alpha of 0.1% and power of 90% (multiple markers were tested, giving similar or smaller group; TNF-α – 58 subjects). 87 subjects/group for a 25% difference in CD8+CD25+ T cell (α=0.1%, power - 90%). These analyses take into account multiple comparisons which are foreseen for immune signature determination.
We believe that these projected effect sizes would potentially be clinically meaningful and credible.

7. Study Closure or Definition of End of Trial

The study will end when:

- Final participants attend last study visit

OR

i. The planned sample size has been achieved;
ii. There is insufficient funding to support further recruitment, and no reasonable prospect of additional support being obtained;
iii. Recruitment is so poor that completion of the trial cannot reasonably be anticipated.

8. Data Handling

8.1 Case Report Forms / Electronic Data Record

An electronic case report form (e-CRF) will be used to collect study data. The e-CRF will be developed by the study data centre at the CASTOR EDC (https://castoredc.com) and access to the e-CRF will be restricted, with only authorised site-specific personnel able to make entries or amendments to their Participants' data. It is the investigator's responsibility to ensure completion and to review and approve all data captured in the e-CRF.

All data handling procedures will be detailed in a Study Specific Data Management Plan. Data will be validated at the point of entry into the e-CRF and at regular intervals during the study. Data discrepancies will be flagged to the study site and any data changes will be recorded in order to maintain a complete audit trail (reason for change, date change made, who made change).

8.2 Record Retention

As discussed above in patient recruitment procedures, subjects will be initially identified by a member of the patient's existing clinical care team who has access to patient records in relation to clinical service. They will inform the patient about the possibility of participating in a study.

Patient data will be pseudo-anonymised with a study number given to the patient. This study number will be used for blood tubes. To enable evaluations and/or audits from regulatory authorities, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records), all original signed informed consent forms, in accordance with ICH GCP, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. Data will be retained at the Data Centre for a minimum of 10 years.

Only the Chief Investigator, who is a member of the direct care team, will have access to the participants' personal data during the study. These procedures will comply with the 1998 Data Protection Act. Biological samples collected from participants as part of this study will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities will meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

Personal data will be stored on NHS password protected computers, which are secured as per local NHS protocols. No personal patient details will be on the University computers.

Manual files with personal information will be kept in a secure location in the Clinical Research Facility, containing copies of the consent forms and a sample collection record.

Only members of the study team will have access to any of the data generated.

To enable evaluations and/or audits from regulatory authorities, the data will be retained at the Data Centre for 15 years. The data records will include including the identity of all participating subjects, (to ensure the subjects information can be linked to records), all original signed informed consent forms, source documents, and detailed records of treatment disposition in accordance with ICH GCP, local regulations.

9. Protocol Amendments

Any change in the study protocol will require an amendment. Any proposed protocol amendments will be initiated by the principle investigators and any required amendment forms will be submitted to the regulatory authority, ethics committee and sponsor. The principle investigators will liaise with study sponsor to determine whether an amendment is non-substantial or substantial. All amended versions of the protocol will be signed by the principle investigator and Sponsor representative. Before the amended protocol can be implemented favourable opinion/approval must be sought from the original reviewing Research Ethics Committee and Research and Development (R&D) office(s).

10. Ethical Considerations

10.1 Ethical conduct of the study

The study will be carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and its revisions (Tokyo [1975], Venice [1983], Hong Kong [1989], South Africa [1996] and Edinburgh [2000]).
Favourable ethical opinion will be sought from an appropriate REC before Participants are entered into this clinical trial. Participants will only be allowed to enter the study once either they have provided written informed consent
The principle investigator will be responsible for updating the Ethics committee of any new information related to the study.

10.2 Informed consent

Written consent will be obtained from each trial participant; each participant will receive a signed copy of the consent form. Participants unable to provide written informed consent will be excluded from the study.
The Research Nurse or investigator will explain verbally and in writing the exact nature of the study in writing with the provision of Participant information sheet. This will include the known side-effects that may be experienced, and the risks of participating in this clinical trial. Trial participants will be informed that they are free to withdraw their consent from the study or study treatment at any time.

10.3. Assessment and management of risk

The small amounts of venous blood which will be obtained are safe and do not pose any additional risk. All procedures for venepuncture will be fulfilled to ensure safety of participants and the procedure will be carried out by trained medical practitioner, member of InflammaTENSION team.
The study is classified as Type A = No higher than the risk of standard medical care

10.4. Blinding

Patient data will be pseudo-anonymised with a study number given to the patient. This study number will be used for blood tubes. These numbers will be used for blinding. All investigators performing clinical and laboratory assessment will be blinded towards hypertension status and group allocation of individual subjects.
Only the Chief Investigator and research fellow trained who is a member of the direct care team will have access to the participants' personal data during the study. These procedures will comply with the 1998 Data Protection Act. Biological samples collected from participants as part of this study will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities will meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

11. Insurance and Indemnity

The InflammaTENSION study is sponsored by NHS GGC. The sponsor will be liable for negligent harm.. NHS indemnity is provided under the Clinical Negligence and Other Risks Indemnity Scheme (CNORIS). Non-negligent harm from the protocol, design of the study and which is therefore non-negligent, will be covered by the University of Glasgow.

The NHS has a duty of care to Participants treated, whether or not the Participant is taking part in a research study, and the NHS remains liable for clinical negligence and other negligent harm to Participants under its duty of care.

12. Funding

Funding for the InflammaTENSION study will be from the European Research Council and the BHF Centre of Excellence Award.

Grant Funder: European Research Council and British Heart Foundations

Grant award number: ERC Grant agreement in negotiations; BHF - RE/13/5/30177

13. Dissemination of Findings

The research findings will be disseminated to healthcare professionals and public health specialists through newsletters; to members of the public by newspapers and other media as well as by information sessions; and to the wider scientific community through peer reviewed publications and presentations.

No identifiable participant information will be detailed within the research findings

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