NETSCC, HTA

8th April 2011

TABUL

Temporal artery biopsy vs ultrasound in diagnosis of giant cell arteritis

The Role of Ultrasound Compared to Biopsy of Temporal Arteries in the Diagnosis and Treatment of Giant Cell Arteritis (GCA).

Study Protocol Version 5.0

Study Protocol Date: 31st January 2011

REC Reference Number: 09/H0505/132

Chief Investigator: Dr Raashid Luqmani

Sponsor: University of Oxford

Funder: Health Technology Assessment (HTA)

HTA Reference Number: 08/64/01

Contents

1. PROTOCOL AMENDMENT HISTORY

Version No	Date	Author	Details of change
4	08 Apr 2010	Dr Luqmani (CI)	Change to exclusion criteria (PMR) – Minor – Ethic Approval received 15th April 2010

2. SYNOPSIS

Study title	**The Role of Ultrasound Compared to Biopsy of Temporal Arteries in the Diagnosis and Treatment of Giant Cell Arteritis (GCA).**
Short title	**T**emporal **A**rtery **B**iopsy vs **Ul**trasound in diagnosis of giant cell arteritis (TABUL)
Internal Ref No	REC: 09/H0505/132, HTA: 08/64/01
Study Design	Cohort study (Observational)
Number of Participants	430 (402 to be recruited onto study)
Primary Objectives	**1.** To evaluate the diagnostic accuracy (sensitivity and specificity) of ultrasound as an alternative to temporal artery biopsy for the diagnosis of GCA in patients referred for biopsy with suspected GCA. **2.** To evaluate the cost-effectiveness (incremental cost per QALY) of ultrasound instead of biopsy in the diagnosis of GCA.
Secondary Objectives	**3.** To evaluate inter-observer agreement in the assessment of ultrasound and temporal artery biopsy. **4.** To elicit expert views on the appropriateness of performing a biopsy following ultrasound using clinical vignettes. **5.** To evaluate the diagnostic accuracy (sensitivity and specificity) of the sequential diagnostic strategy from **4** as an alternative to temporal artery biopsy alone in the diagnosis of GCA. **6.** To evaluate the cost-effectiveness (incremental cost per QALY) of the diagnostic strategy from **4** instead of biopsy alone in the diagnosis of GCA.

3. ABBREVIATIONS

AE	Adverse Event
CI	Chief Investigator
CTRG	Clinical Trials & Research Governance, University of Oxford
DPA	Data Protection Act
GCP	Good Clinical Practice
GCA	Giant Cell Arteritis
HTA	Human Tissue Act
NIHR HTA	NIHR Health Assessment Technology (Funder)
ICF	Informed Consent Form
NDORMS	Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences
NIHR	National Institute for Health Research
NOC	Nuffield Orthopaedic Centre
NRES	National Research Ethics Service
PI	Principle Investigator
OMB	Oxford Musculoskeletal Biobank
PIS	Participant/Patient Information Sheet
R&D	NHS Trust Research and Development Department
REC	Research Ethics Committee
RN	Research Nurse
SAE	Serious Adverse Event

SOP	Standard Operating Procedure
TABUL	Temporal artery biopsy vs. ultrasound in diagnosis of giant cell arteritis
TABUL Office	TABUL Co-ordinating centre, NDORMS, Oxford
US	Ultrasound

4. TABUL STUDY PERSONNEL

4.1 Chief Investigator:

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4.4 Co-applicants

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John Salmon (JS), Ophthalmology Department, John Radcliffe Hospital, Oxford
Richard Wakefield (RW), Rheumatology Department, University of Leeds
Eugene McNally (EM), Radiology Department, Nuffield Orthopaedic Centre (NOC)
William Hamilton (WH), Department of Primary Care, University of Bristol
Brendan McDonald (BM), Pathology Department, John Radcliffe Hospital, Oxford
Konrad Wolfe (KW), Pathology Department, Southend University Hospital
Colin Pease (CP), Rheumatology Department, Leeds General Infirmary
Wolfgang Schmidt (WS), Medical Center for Rheumatology, Berlin-Buch

4.5 Investigators involved in future sub-studies (blood and tissue analysis)

1. Dr David Greaves, Reader in Pathology at the Sir William Dunn School of Pathology, University of Oxford
2. Professor David Dewhurst, Director of Learning Technology, University of Edinburgh, Learning Technology Unit
3. Dr Ann Morgan, HEFCE Clinical Senior Lecturer Biomedical Research Unit, University of Leeds
4. Dr Nick Platt, Weatherall Institute of Molecular Medicine, University of Oxford
5. Dr Raashid Luqmani, Consultant/Senior Lecturer in Rheumatology, Botnar Research Centre, University of Oxford

5. TABUL OBJECTIVES

Short title of study: Temporal **A**rtery **B**iopsy vs **Ul**trasound in diagnosis of giant cell arteritis (TABUL)

Estimated Number of Centres: Greater than or equal to 25 centres

5.1 Planned investigation:

5.1.1 Research Objectives

Primary objectives:

1. To evaluate the diagnostic accuracy (sensitivity and specificity) of ultrasound as an alternative to temporal artery biopsy for the diagnosis of GCA in patients referred for biopsy with suspected GCA.
2. To evaluate the cost-effectiveness (incremental cost per QALY) of ultrasound instead of biopsy in the diagnosis of GCA.

Secondary objectives

3. To evaluate inter-observer agreement in the assessment of ultrasound and temporal artery biopsy.

4. To elicit expert views on the appropriateness of performing a biopsy following ultrasound using clinical vignettes.

5. To evaluate the diagnostic accuracy (sensitivity and specificity) of the sequential diagnostic strategy from **4** as an alternative to temporal artery biopsy alone in the diagnosis of GCA.

6. To evaluate the cost-effectiveness (incremental cost per QALY) of the diagnostic strategy from **4** instead of biopsy alone in the diagnosis of GCA.

5.2 Deliverables

As well as the final Health Technology Assessment (HTA) report on the role of ultrasound vs. biopsy in the diagnosis of GCA, we expect to produce the following: original articles in peer reviewed journals on the pathology of GCA; ultrasound appearances in GCA; comparative value of ultrasound compared to biopsy in GCA; a website of ultrasound exemplars correlated with temporal artery biopsy exemplars which could be used for future training; a biobank of temporal artery biopsies, together with serum, plasma RNA and DNA for future planned studies of GCA (outlined in Appendix I) to add value to the current study by making use of this invaluable resource.

5.3 Existing research

Giant cell arteritis is a common form of vasculitis in older persons[1] affecting 220 patients/million/annum in the UK[2] which specifically involves large arteries particularly the branches from the aorta towards the head and neck. Early diagnosis and treatment are important because patients may develop significant complications including blindness and stroke[3]. Irreversible visual loss occurs in up to 20% of patients[4]. There is no true gold standard for the diagnosis of the condition. Temporal artery biopsy is performed in almost every case but the diagnostic yield from this test can be as low as 66%[5]. The specificity of biopsy is less than 100% since other forms of vasculitis may present with the same pathological features[6]. The role of biomarkers such as CRP, ESR and circulating serum cytokines in the diagnosis of giant cell arteritis remain unsatisfactory[7]. A careful history and clinical examination are helpful but some of the key features that distinguish patients with giant cell arteritis such as the presence of jaw or tongue claudication occur in less than 20% of patients[4]. The American College of Rheumatology have developed classification criteria for GCA but these are primarily designed to distinguish one form of vasculitis from another form of vasculitis rather than distinguish patients who have headache of a non-specific nature from those who specifically have the condition of GCA[1]. There are no diagnostic criteria for GCA. Ultrasound imaging and high resolution MRI scanning of temporal arteries are equivalent, non invasive techniques for the investigation of suspected GCA[8]. The current use of ultrasound as a diagnostic tool for GCA is limited. Only 2 of the identified recruiting centres have actually started using the technique, largely due to the uncertainty of benefit to patients.

In this proposal we will assess the value of ultrasound examination of temporal arteries as an adjunct to diagnosis of GCA in addition to its potential role as a substitute for temporal artery biopsy. Ultrasound examination of temporal arteries is non invasive and does not involve ionizing radiation. It can provide information about the vessel wall throughout the length of the vessel and potentially can evaluate the presence of skip lesions which are a significant problem in histological examination[9,10]. Ultrasound assessment with a colour duplex may demonstrate the “halo sign” which is defined as a dark area around the vessel lumen and is thought to be due to vessel oedema in the wall[9]. Additional scanning of axillary arteries substantially increases the diagnostic yield[11]. A small study from Jerusalem[12] reported a positive predictive value of the halo sign of only 50% but a negative predictive value of 96%. In a meta-analysis of the test performance of ultrasonography in giant cell arteritis[5], the sensitivity was reported to be 69% with the specificity of 82% for the halo sign as compared with temporal artery biopsy. However, it is important to bear in mind that the value of ultrasound depends on the pre-test probability of the patient having giant cell arteritis. If the probability is 10% or less and an ultrasound is negative this can effectively exclude the condition and thus avoid the morbidity and cost of a biopsy. In patients in whom the ultrasound reveals bilateral halos the specificity is reported to be 100%[5] making biopsy unnecessary. In a small study by Karahaliou et al[13], 55 patients underwent colour Doppler ultrasound for suspected temporal arteritis, producing sensitivity and specificity values of 82% and 91% respectively, suggesting that the scan could be used in place of a biopsy for some patients. Ultrasonography of the temporal arteries is highly operator dependant and it is important to develop expertise with the technique before applying it. Therefore adequate training is essential. We are not aware of any previous ultrasound studies involving multiple centres. The current study design is able to demonstrate the place for ultrasound testing of temporal arteritis in secondary care settings in the UK, therefore showing its potential for widespread applicability.

5.4 Research methods

5.4.1 Overall design

The overall design consists of a cohort study of 402 participants with suspected GCA who will be followed up for 6 months; a cost-effectiveness study comparing ultrasound with temporal artery biopsy; a study of observer agreement in evaluating ultrasound and temporal artery biopsies; and an expert panel assessing the appropriateness of alternative strategies for diagnosing and treating patients with suspected GCA.

5.4.2 Cohort study design - to evaluate the diagnostic accuracy of ultrasound and biopsy

The cohort study will address study objectives **1** and **5**:

1. To evaluate the diagnostic accuracy (sensitivity and specificity) of ultrasound as an alternative to temporal artery biopsy for the diagnosis of GCA in patients referred for biopsy with suspected GCA.

5. To evaluate the diagnostic accuracy (sensitivity and specificity) of the sequential diagnostic strategy from **4** as an alternative to temporal artery biopsy alone in the diagnosis of GCA.

The proposed study will recruit a cohort of eligible patients (predominantly from primary care referrals) suspected to have new onset of GCA and who would routinely have a temporal artery biopsy. There is no therapeutic intervention in the study. Patients will be referred for urgent evaluation and treatment. Conventional treatment is with high dose glucocorticoids, and the recommended management requires a subsequent temporal artery biopsy in order to substantiate the clinical diagnosis[3]. Consenting patients will be recruited as soon as possible after initial referral (**Study Visit 1** - approximately 60-90 minutes). Patients will be separately consented to store blood and artery biopsy samples for subsequent cohort studies outlined in Appendix I. The additional investigation (ultrasound scanning) will be performed but the results will not be made available to the clinician in charge of the patient's care, so that they cannot influence current diagnosis and management. The patient will be reviewed after 2 weeks (**Study Visit 2**) to assess the clinical state, take a further blood sample, interpret the biopsy findings and reconsider the diagnosis and management (**Study Visit 2** - approximately 45-60 minutes) The patients will be followed up according to local practice, but will all have a study follow-up assessment and final blood sample taken after 6 months (**Study Visit 3** - approximately 45-60 minutes).

5.4.3 Cost-effectiveness of ultrasound in comparison with biopsy

The cost-effectiveness study will address objectives **2** and **6**:

2. To evaluate the cost-effectiveness (incremental cost per QALY) of ultrasound instead of biopsy in the diagnosis of GCA.

6. To evaluate the cost-effectiveness (incremental cost per QALY) of the diagnostic strategy from **4** instead of biopsy alone in the diagnosis of GCA.

The value of a diagnostic test depends not only its ability to correctly identify patients with and without the condition, but on the costs and health consequences of failing to make a correct diagnosis. The latter element of this assessment cannot be estimated directly from the proposed cohort study. We propose to evaluate the costs of delivery of the alternative diagnostic strategies and the impact of high dose steroid treatment and biopsy on health related quality of life within the patient group selected for the cohort study. A decision analytic model will be developed and other values required to populate it will be derived from the expert panel's assessment of appropriateness and systematic review and synthesis of existing literature.

A decision analytic model will be developed in order to estimate the long term costs and effects of the alternative diagnostic strategies. The model will draw on the analysis of the patient level data to distinguish the potential differences in costs between strategies, the potential benefits of avoiding the invasive biopsy and rapid discontinuation of steroid therapy. The results from the expert panel will be used to model the likely impact on treatment and management of patients according to the results of the alternative diagnostic strategies. The long term impact of those decisions will be incorporated by systematic review and synthesis of existing literature. Parameters that will be required for the model include the probability of GCA complications with and without treatment, their associated costs and

impact on health related quality of life. We will develop this model in the first year of the project in order to help prioritize those parameters that are likely to be important drivers of cost effectiveness.

5.4.4 Establish a central resource of ultrasound and biopsy images and assess variability in interpretation

To address the issue of generalisability and the suggestion of a central review and validation of recorded ultrasound examinations, we propose establishing a central web-based resource of ultrasound and biopsy images based in Oxford. The resource will serve three purposes:

(i) as a means of quality control (see sections 6.1 and 6.2) of ultrasound and biopsy performance and interpretation;

(ii) to develop a web-based survey for assessing inter- and intra-observer agreement in interpreting ultrasound and biopsy; and

(iii) a future education and training resource subject to ethical approval.

For each of ultrasound and biopsy we plan to recruit 20-30 clinicians from the study collaborating centres to interpret images according to the study protocol via a secure web-based assessment tool. The results of these analyses will allow an assessment of variability in the interpretation of both ultrasound and biopsy that can also be used in the statistical modeling of diagnostic accuracy and cost-effectiveness.

5.4.5 Use of an expert panel to assess the appropriateness of performing ultrasound and biopsy using clinical vignettes

The cohort design (with biopsy results provided but ultrasound results hidden from clinicians) ensures that clinical decisions (e.g. continuation or withdrawal of steroids) are based on current standard practice. Key questions that the cohort design cannot address are (i) the impact of ultrasound results on the appropriateness of a subsequent biopsy to aid diagnosis; and (ii) the impact of ultrasound results and/or biopsy results and clinical response on the diagnosis and decisions to continue/withdraw steroid treatment. To address these questions we propose developing clinical vignettes based on cohort participants and the research literature to obtain clinicians' views on the appropriateness of different diagnosis and treatment options at different stages of the disease. This would involve a 9-15 person expert panel (comprising rheumatologists, ophthalmologists, and general practitioners) based on the RAND/UCLA Appropriateness Method[14], rating appropriateness individually in round 1 using a secure web-based survey, followed by a convened meeting and individual round 2 rating. The results will provide an assessment of consensus and variability amongst clinicians that can be incorporated into statistical modeling, e.g. for sensitivity analysis and predicting outcomes under hypothesized diagnostic and treatment strategies. A key output of this component is the sequential diagnostic strategy to be evaluated in research objectives **4** and **5**.

4. To elicit expert views on the appropriateness of performing a biopsy following ultrasound using clinical vignettes.

5. To evaluate the diagnostic accuracy (sensitivity and specificity) of the sequential diagnostic strategy from **4** as an alternative to temporal artery biopsy alone in the diagnosis of GCA.

6. TABUL TRAINING

The TABUL office will hold training days as required for key members of the study teams (e.g. research nurses, ultrasonographers and pathologists).

6.1 TABUL Ultrasound (US) training and review

It is essential for the TABUL study that we have robust training programme to assess the ultrasound (US) technique and data interpretation. The training programme must ensure adequate standards observer agreement is achieved for each ultrasonographer.

For each of the TABUL centres:

(i) Will be provided with a training pack from Dr Wolfgang Schmidt,

(ii) Complete assessment of 10 training cases (patients or staff members) of similar age and gender to the study cohort. All training participants will be screened and consented prior to the ultrasound scan. Subsequently, all ultrasonographers will submit their scan images, which will be reviewed by the expert radiology panel.

(iii) Complete a video assessment to identify normal and abnormal US scans for GCA.

(iv) Complete a 'hands-on' assessment of a patient with active GCA prior to biopsy ('hot' case training).

The main purpose of this is to familiarise the observers with the technique, and to ensure that they can recognise normal and atherosclerotic vessels.

6.2 TABUL Biopsy review

During the course of the study biopsy sample suitability and histology reporting will be reviewed as part of quality assurance (QA) and quality control (QC).

7. SUB STUDIES (SUBJECT TO FURTHER FUNDING)

NOTE: SUB STUDIES WILL ONLY BE UNDERTAKEN AS SEPARATE STUDIES IF FUNDED AND GIVEN ETHICAL APPROVAL

7.1 Evaluation of immune abnormalities in GCA

Aims & Objectives

1) To determine the number and phenotype of macrophages in temporal artery biopsy samples.
2) To determine the level of inflammatory cytokines and other plasma markers in whole blood, plasma and serum samples.
3) To determine the bioactivity of chemokines in whole blood, plasma and serum samples.
4) To prepare mRNA from tissue samples and whole blood samples.

Methods

1) Temporal artery biopsy samples will be stored frozen in OCT or fixed and embedded in paraffin. Sections will be prepared with a microtome and stained with a range of antibodies to detect specific cell types. Antibodies to include some or all of the following: smooth muscle cell actin, CD3, CD4, CD8, CD31, CD45, CD68, Galectin-3, Lys6C, Gr1, von Willebrand Factor, MOMA2, Fractalkine, CCL2,CCl3, CCL5, chemerin, TNF, CCR1, CCR2, CCR5, CX3CR1, ChemR23.

2) Whole blood, plasma and serum samples will be analyzed for expression of a panel of inflammatory cytokines by ELISA or related methodologies (e.g. Luminex) including the following: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL17, IL-23, M-CSF, GM-CSF, Fractalkine, CCL2, CCL3, CCL5, chemerin, TNF.

3) Plasma and serum samples will be used in bioassays for specific leukocyte receptors. Bioassays will include DiscoveRx PathHunter and cAMP assays, chemotaxis assays using modified Boyden chambers and monocyte adhesion assays.

4) Total RNA and polyA+ mRNA will be prepared from whole blood and temporal artery samples by standard methods and used for RT-PCR analyses and gene array studies.

7.2 Assessment of inflammatory mediators in situ in GCA

Aims & Objectives

1) To determine the number and phenotype of lymphocytes in temporal arteritis.
2) To determine the role of matrix metalloproteinases in temporal arteritis.

Methods

1) We will use a combination of techniques (immunohistochemistry, RT-PCR, ELISA, Western blotting) to assess the tissue distribution of a number of key inflammatory cells and cytokines and other cytokine related proteins that may be relevant to the pathogenesis of GCA.

2) Temporal artery biopsy samples will be stored frozen in OCT or fixed and embedded in paraffin. Sections will be prepared with a microtome and stained with a range of antibodies to detect specific

cell types. Antibodies to include some or all of the following: TNF related proteins such as TSG6, and LIGHT, and a range of matrix metalloproteinases.

3) Whole blood, plasma and serum samples will be analyzed for expression of TNF related proteins such as TSG6, and LIGHT, and a range of matrix metalloproteinases.

7.3 Assessment of inflammasome in GCA

Aims and objectives

1) To determine the extent of inflammasome activation in circulating cells and cells from temporal artery specimens in GCA.
2) Measurement of apoptosis and pyroptosis in circulating cells and tissue derived cells in patients with giant cell arteritis.
3) Assess the profile and phenotype of T suppressor phenotypes (such as myeloid suppressor cells) in the tissue samples.

Methods
1) Temporal artery biopsy samples will be stored frozen in OCT or fixed and embedded in paraffin. Sections will be prepared with a microtome and stained with a range of antibodies to detect specific cell types. Antibodies to include some or all of the following: NALP 1, 2 and 3, CASPASE 1, 3, 5 and 11, IL1 beta.

2) Whole blood cells will be assessed for the expression of inflammasomes by ELISA or related methodologies: NALP 1, 2 and 3, CASPASE 1, 3, 5 and 11, IL1 beta.

3) Detection of DNA fragmentation in tissue cells and circulating cells by gel electrophoresis.

4) Tissue histology, immunostaining of sections and FACS analysis of cells from biopsies for T cell markers - CD3,CD4, CD8 and their subtypes, including CD14; suppressor phenotypes (T regs and myeloid suppressor cells).

5) Functional assays of function of cells derived from tissue biopsies in vitro (including suppression of T cell proliferation).

7.4 Development of a training programme using images obtained from histology and video of ultrasound scans

Aims and objectives

1) The clinical data collected during this study has the potential to form the basis for high quality educational materials for both postgraduate (radiographers, pathologists, rheumatologists) and undergraduate learners.

2) We propose to develop the material assembled as a small number of virtual patient scenarios to test the effectiveness of a training programme to enhance the diagnostic ability of undergraduates and postgraduates in ascertaining diagnosis of GCA.

Methods

1) Fully anonymised data would be linked to illustrative examples of clinical scenarios (virtual patients) to be used for teaching and training. The development of the educational materials will be subject to additional funding.

2) All patients in the current study will be asked to complete and sign informed consent forms agreeing to the use of their fully anonymised data for educational purposes (paper-based, electronically-mediated, web-based) to a global audience of clinicians and non-clinicians.

3) We will measure the impact of a training programme using the stored images and clinical scenarios in the diagnostic accuracy and decision making.

4) We will test the validity of an On-line atlas of histological features covering the spectrum of findings in temporal artery biopsies to standardize reporting of biopsies.

5) We will test the validity of an On-line atlas of ultrasound features covering the spectrum of findings in temporal artery and axillary artery scans to reporting of scans.

8. PLANNED INTERVENTIONS

This is a study of diagnostic test accuracy. The two interventions (index tests) being compared are high resolution ultrasound and temporal artery biopsy. All participants will have ultrasound followed by temporal artery biopsy. The reference standard diagnosis against which the index tests will be evaluated is described in Figure 1 (algorithm for patients). Detailed protocols for performing and reporting ultrasound and biopsy will be developed.

High resolution ultrasound is defined as a grey scale frequency of $\geq$ 10 MHz, and a colour frequency of > 6 MHz. The axial and the lateral resolution should be at least 0.3 mm. Duplex ultrasound scans of both temporal and axillary arteries will be performed according to pre-defined protocols[9,11]. For the temporal artery examination, we will follow the common superficial temporal artery with the parietal and frontal branch in two planes on both sides with colour Doppler ultrasound. In case of turbulence and persistence of blood flow in diastole, we will perform pulse wave Doppler. A stenosis is defined as an increase of the maximum flow velocity of more than two fold compared to an area proximal or distal to this point[9].

The scans will be recorded and the video and still images obtained from both TABUL training cases and recruited participants. All scans will be reported at the time of acquisition and the results sent to the TABUL study co-ordinator at the TABUL Office; digital video clips plus cross sectional still images from each patient will be uploaded to a central secure server sited in Oxford. Patients and treating physicians will not be told the results of the scan. All images will be reviewed by an expert radiology panel (EM, BD, RW and WS) every 6 months; in addition, a result in which there is uncertainty will be reviewed for technical expertise and to assess the presence of abnormality if it is possible to do so. Scan results will be reported according to the number of halos, stenoses or occlusions at each of the 4 arteries sampled, along the length of each vessel according to a standardised sampling protocol[9,11].

Temporal artery biopsies will be obtained according to local practice but sites will be encouraged to follow British Society for Rheumatology (BSR) guidelines[15], with a biopsy length of at least 1 cm. Slides will be prepared, including stains for haematoxylin and eosin, and any other stains such as van Gieson according to local practice. Interpretation of the biopsy will be according to BSR guidelines, recording the presence of giant cells, intimal hyperplasia, reduplication of the internal elastic lamina, transmural inflammation, occlusion and the presence of small vessel change (defined as the presence of aggregates of mononuclear cells surrounding a capillary, distant from an uninflamed temporal artery). The pathologists will report findings according to these criteria as positive, negative or intermediate. Patients will be treated in the normal way, with high dose steroid continued until the biopsy results are available, when a clinical decision will be made (at **Study Visit 2**, approximately 14 days after the biopsy) regarding the diagnosis and treatment. The purpose of the final study visit 6 months after enrolment is to determine the certainty of the original diagnosis in light of any new clinical developments, such as the emergence of an alternative diagnosis.

At each of the 3 study visits, we will take a blood sample to test the acute phase response (CRP and ESR) as part of standard care. In addition, with the patient's consent, at each of the study 3 visits, we will take following blood samples (maximum total of 85 ml) which will be stored at the Oxford Biobank:

(i) **Study Visit 1** - approx. 35 ml total (10 ml EDTA whole blood and plasma and serum samples)
(ii) **Study Visit 2** - approx. 25 ml total (plasma and serum samples)
(iii) **Study Visit 3** - approx. 25 ml total (plasma and serum samples)

9. PLANNED INCLUSION/EXCLUSION CRITERIA

We will adopt a pragmatic approach to recruitment, i.e. aim to include all patients undergoing temporal artery biopsy for suspected GCA. A lower age restriction of 18 will be applied although no age criteria is necessary for this disease type as we expect the majority of patients to be elderly. The clinicians will be using their judgment and clinical experience to determine whether or not to refer for biopsy. We will include patients with pre-existing polymyalgia rheumatica. Most patients will be treated with a standard dose of prednisolone, but some may be commenced on prednisolone plus another immunosuppressive agent. They will be included even though we suspect that the biopsy and scan results may be affected differently than when compared to steroids alone.

9.1 Inclusion criteria

For the cohort study

(1) A clinical suspicion of new diagnosis of GCA e.g. patients with a new onset of headache, scalp tenderness, with or without elevated CRP or ESR, jaw or tongue claudication with or without visual loss.

(2) The clinician decides that the patient requires an urgent temporal artery biopsy to determine whether or not the diagnosis is GCA.

(3) The patient agrees and provides NHS consent to undergo a temporal artery biopsy as part of standard care.

(4) Patients have been started on high dose glucocorticoids or will be started on high dose glucocorticoids.

(5) Patients must be willing to attend for an ultrasound scan of their temporal and axillary arteries.

(6) Participants must be willing to give informed written consent or willing to give permission for a nominated friend or relative to provide written informed assent if they are unable to do so because of physical disabilities e.g. sudden onset of blindness/vision loss which can be caused by GCA (this will be made clear in the ethics approval application).

(7) Must be 18 years of age or over.

For the training cases

(1) Patients attending hospital outpatient or in patient departments for assessment for any condition (apart from giant cell arteritis or polymyalgia rheumatica) or healthy staff volunteers.

(2) Above the age of 50 years.

(3) Willing to attend for an ultrasound scan of their temporal and axillary arteries.

(4) Willing and able to give written informed consent.

9.2 Exclusion criteria

For the cohort study

(1) Previous diagnosis of GCA.

(2) Use of high dose glucocorticoid (>20mg prednisolone/day) for management of current suspected GCA for more than 7 days prior to the dates of the ultrasound and biopsy.

(3) Long term (>1 month) high dose (>20mg per day at any time) steroids for conditions other than PMR, within three months prior to study entry.

(4) Inability to give informed consent (either written consent or verbal assent from a relative or carer)

(5) Inability to undergo an ultrasound scans of the temporal and axillary arteries.

(6) Patients with a known cause of headache (not due to GCA), or any condition which would preclude the need for a temporal artery biopsy.

(7) Patients who are unable to undergo an ultrasound scan and a temporal artery biopsy within 7 days of starting glucocorticoids.

For the training cases

(1) Diagnosis of suspected GCA or a previous history of diagnosed or suspected GCA.

(2) Inability to give written informed consent.

(3) Inability to undergo an ultrasound scans of the temporal and axillary arteries

10. ETHICAL ARRANGEMENTS

All patients recruited to the study will be incident cases with most being referrals from primary to secondary care. This study will meet the NIHR criteria for portfolio research and will therefore involve a full IRAS application with the support of the Thames Valley CLRN. Approval to conduct the study will be sought from each local Trust via Site Specific Information (SSI) Forms.

Potential participants will be approached by a member of the research team, and will be provided with an Participant Invitation Study Letter and Participant/Patient Information Sheet (PIS) and given the opportunity to discuss the research project prior to obtaining informed consent or assent. Verbal consent will be obtained from all clinicians responsible for the care of the potential participant in the first instance prior to approaching their patient. We foresee that potential participants will be identified following referral from Primary Care for assessment by the on-call rheumatologist. We will fully inform the on-call rheumatologists about the study; we will establish weekly contact with them and provide them with a Participant/Patient Information Sheet (PIS) for them to give to all potential participants. The PIS will inform the potential participant that a member of the research team, mainly the research nurse, will contact them to discuss the study in more detail. The PIS will welcome the potential participant to contact the research team to inform them of their decision to participate or not. Due to the nature of the study which is as a result of the requirement to undertake the ultrasound scan prior to the biopsy (both being required within 7 days of the participant commencing glucocorticoid therapy). An example of the 7 day timeframe is given below:

Time and Date participant started glucocorticoid therapy (Day 0):	10.30am on Monday 7th February 2011
7 days deadline (Day 7): Within these days, Visit 1, ultrasound and biopsy need to be completed before the date opposite.	10.30am on Monday 14th February 2011

No study related activity will be performed prior to obtaining fully written informed consent or assent. The intervention is a non invasive procedure which does not pose any additional risks, discomfort or anxiety to the patient and every effort will be made to ensure the participants are not caused undue anxiety.

Consent will be obtained for the use of anonymised biopsy and scan images from this study as a teaching tool. The participants will also be asked to give consent for their information to be held by the research team indicating that they are willing to be approached for involvement in future ethically approved GCA research. Participants will also be asked to consent to the use of their data and tissue collected as part of this study for future GCA research through the creation of a Biobank which would utilize blood, DNA and biopsy samples (see Appendix I). The creation of this Biobank will conform to all the requirements in place through the Data Protection Act (DPA) and Human Tissue Act (HTA) including procedures for a Data Custodian who would be responsible for setting up procedures to enable access to this data. Any future research not specified within this application would involve a separate ethical and funding application. If patients need to attend for a separate visit we will pay their transport costs.

To consider the question of research equipoise, we surveyed the proposed study centres to discover if any are already using ultrasound as part of the standard diagnostic workup. In 2 centres, ultrasound scanning of temporal arteries is being used, but the centres involved have accepted the study requirements, so that investigators will not be made aware of the scan results for any individual patient

11. RISKS AND BENEFITS

The use of a cohort design that reflects current practice reduces additional study risks to participants. We have identified two risks:

(1) The potential for introducing a delay in performing a temporal artery biopsy in the appropriate time frame of within 7 days of starting glucocorticoid therapy, thereby leading to an increase in the number of false negative biopsies. This might result in a higher level of uncertainty for the clinician who has to decide whether or not to continue long term high dose glucocorticoid therapy. We do not anticipate this to be a germane issue; if there is any delay in obtaining the scan, we would exclude the patient from the study. In fact we anticipate that for the purpose of the study, we will have to streamline the clinical service to improve the links between rheumatologists and the biopsy service to reduce delay in investigating suspected cases of GCA. Patients and physicians may feel less certain about the diagnosis, if based on ultrasound assessment alone. This is not a risk in the current study since all patients will have conventional investigations. For cases of diagnostic uncertainty, in whom the clinical suspicion for a new diagnosis of GCA is intermediate, the patients may need to undergo both the ultrasound scan and a biopsy in order to improve the diagnostic accuracy. The effect of ultrasound results instead of or as well as a biopsy will be tested in a non-real setting, but based on the real cases recruited from the study

(2) The potential for glucocorticoid therapy to be withdrawn in a biopsy-negative participant but the hidden ultrasound indicates strong evidence of GCA. In all biopsy-negative patients we will check the scan; if it is positive we will highlight this to the clinician immediately after the patient has been evaluated at the 2 week visit (the point at which treatment may be significantly reduced if the biopsy is negative). The clinician can change the diagnosis and treatment, but the patient would continue in the study. Data will be analyzed on an intention to treat basis. The number of these events will be recorded by the study coordinator and reviewed by the data monitoring committee.

Participants will be fully informed of the risks of the study. The information will be included in the Participant/Patient Information Sheet (PIS) and will be reiterated during the consent process. Participants will be fully informed about the study prior to enrolment. We plan for a member of the research team, mainly the research nurse, to contact potential participants by telephone to discuss the study. This will only be done after liaising with the on-call rheumatologist and ensuring that a Participant Invitation Study Letter has been given to all potential participants. We plan for the member of the research team to contact the potential participant within 72 hours of them commencing glucocorticoid therapy. In case of an emergency, where potential participants are unable to give written consent (for example if they have suffered sudden visual loss as a result of the suspected GCA) we would seek fully written informed assent from a nominated relative or friend. Patients will receive medication as part of their standard care; the study itself does not involve any medication. Study documentation will be stored for at least 5 years.

A member of the research study team, mainly the research nurse, will also identify and approach potential participants during routine or emergency outpatient clinics. They would provide the potential participant with the Participant Invitation Study Letter and Participant/Patient Information Sheet (PIS) and will discuss the study in more detail and to ascertain whether the potential participants wishes to participate in the study or not. We will normally offer the potential participant 24 hours to consider the study. However, we would in certain circumstances, for example (i) in an emergency to minimise delay in normal care (i.e. performing the biopsy), (ii) where sites are able to provide a fast turnaround time for performing the biopsy, we will offer the opportunity for participants to provide fully written informed consent in less than 24 hours from receiving the information about the study. Time would be made available at clinic to discuss the study in more detail if the potential participant would like to. Arrangements will be made to book the ultrasound and biopsy as soon as possible (within 7 days of commencing glucocorticoid therapy). Written informed consent maybe obtained on the day of the ultrasound scan to minimize participant visits to the hospital. We will also have to take into account the short window period of 7 days following commencing of glucocorticoid therapy. If the participant wishes to attend the hospital at a different time/day to their arranged ultrasound scan to go through the consent process then this will be arranged and their travel costs will be reimbursed; if this falls outside the 7 day window period then the participant will be unable to participate in the study.

12. TABUL STUDY - PROPOSED SAMPLE SIZE

A sample of 402 patients provides 90% power at a 5% type I error rate to test the joint hypothesis that

(i) ultrasound has greater sensitivity than biopsy (to detect an increase in sensitivity from 76% for biopsy (assuming a 0.24 false negative fraction based on 9-44% biopsy-negative GCA) to 87% sensitivity for ultrasound[5]; and

(ii) to detect specificity of ultrasound of no less than 0.83 based on an expected specificity of 0.96[5]. This sample size will allow estimation of a one-sided rectangular confidence region for ultrasound false and true positive fractions, assuming 80% prevalence of GCA in patients having a biopsy for suspected GCA, with the sample size inflated (gamma=0.1) due to uncertainty in the proportion of cases/controls in a cohort design[16].

In order to allow for losses to follow-up we plan to recruit 430 participants to the study. We anticipate that most losses to follow-up will occur in recruited participants not having both ultrasound and biopsy at their appointed time within 7 days of starting steroids. Previous experience of observational studies in PMR[17] suggest no further losses at the week 2 assessment and 4% at the 6 month assessment. However, the primary outcome (reference standard diagnosis) can be derived in participants who do not complete the 6 month assessment. These assumptions will be checked using early monitoring of recruitment and follow-up rates, with recruitment targets modified as necessary.

12.1 Statistical analysis

The analyses will be performed in accordance with a detailed analysis plan developed during the study that meets the reporting requirements of the STARD statement[18]. The primary analyses will estimate:

(i) the sensitivity of an abnormal ultrasound test (compared with an abnormal biopsy test) for predicting the reference standard diagnosis of GCA; and

(ii) the specificity of an abnormal ultrasound test predicting the reference standard diagnosis of GCA against the null hypothesis that specificity is <83%.

Uncertainty in sensitivity and specificity will be estimated using a joint rectangular 95% confidence interval. No interim analysis is planned. Analysis of inter- and intra-rate agreement of ultrasound interpretation and biopsy interpretation will be assessed using kappa coefficients[19]. Data from the expert panel rating appropriateness of diagnostic and treatment strategies will use the standard RAND/UCLA approach for determining consensus[14] A detailed analysis plan will be prepared to specify all a priori analyses including sub-group analyses (e.g. patients with high or low risk, patients with or without PMR, patients with or without visual symptoms at presentation), analysis by specific components of ultrasound and biopsy results (e.g. positive or negative axillary artery scans) and assessment of positive and negative predictive values of tests.

Cost-effectiveness analysis will estimate the mean incremental costs and QALYs to determine the incremental cost-effectiveness ratio of ultrasound as an alternative to biopsy in the diagnosis of GCA. Unit costs will be drawn from national, published sources where available. We will collect unit costs from the participating trusts where this is not feasible or where national sources may not be ideal. Full consideration of uncertainty will be incorporated into the analyses and presentation of results. Probabilistic sensitivity analysis (PSA) will be used to reflect parameter uncertainty and structural issues will be addressed using simple sensitivity analysis. In undertaking PSA, we will draw on both the analyses of patient level data and the evidence synthesis to appropriately reflect uncertainty and correlations between parameters. Modeling methods will conform to good practice as specified in the NICE 2008 methods guide[20]. We will additionally consider both societal and NHS perspectives for the analyses. We will consider the cost effectiveness for relevant patient subgroups, particularly where this is defined in terms of pre-test probability of GCA.

12.2 Proposed PRIMARY outcome measures

The principal outcome is the reference ('gold-standard') standard diagnosis of GCA. This will be based on a composite of:

1. the ACR's classification criteria[1];
2. alternative diagnoses;
3. incidence of a new diagnosis of PMR; and
4. incidence of a GCA-related adverse event during follow-up (see Figure 1).

The reference standard diagnosis will be derived by algorithm from assessment data following database locking. Components of the ACR's classification criteria can be met at any assessment. Pre-specified alternative diagnoses (which may result in a positive temporal artery biopsy) include Takayasu arteritis, ANCA associated vasculitis, polyarteritis nodosa, cryoglobulinaemic vasculitis and rheumatoid vasculitis. Pre-specified GCA-related adverse events include anterior ischaemic optic neuropathy, polymyalgia rheumatica, tongue infarction. Other alternative diagnoses and adverse events will require justification and approval from a panel of three independent investigators.

Health-related quality of life for the cost-effectiveness analysis will be measured at each assessment using the EuroQol EQ5D[21].

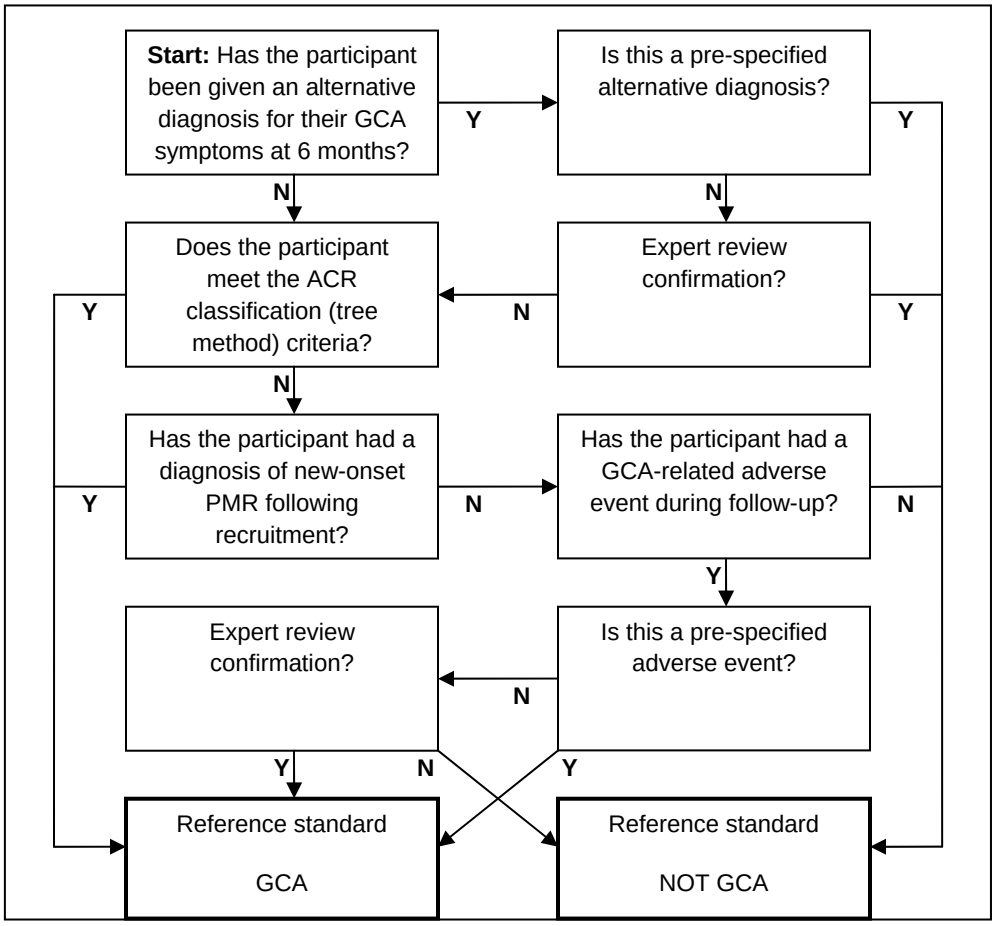


Figure 1 Algorithm for study patients

12.3 Proposed SECONDARY outcomes measures

These will include:

1. Specific adverse events measured at each assessment; daily and cumulative steroid dose; steroid side effects; and pain or dysaesthesia at the biopsy site.
2. Evolution of an alternative diagnosis.At each assessment, the clinician will review the patient looking for evidence of diseases that could explain the presenting symptoms, including the use of the Birmingham Vasculitis Activity Score (BVAS) as a screening tool[22] for other forms of vasculitis.
3. Negative predictive value of ultrasound in preventing the need for temporal artery biopsies.
4. Cost analysis of performing a screening ultrasound examination plus biopsy as part of the diagnostic workup of all patients with suspected GCA; or of performing a screening ultrasound examination instead of biopsy; or of performing a screening ultrasound examination instead of biopsy in cases with a high probability of GCA.
5. Cost analysis of performing a screening ultrasound examination instead of biopsy in cases with a very low probability of GCA as part of the diagnostic workup of all patients with suspected GCA.
6. Prediction of potential harm done to patients by over diagnosis or under diagnosis of GCA as a result of ultrasound use, either alone or in combination with biopsy.
7. Value of axillary artery ultrasound scanning in contributing to the diagnosis of GCA.

8. Analysis of proportion of patients with a biopsy positive halo, stenosis, or occlusion assessed by high resolution ultrasound.
9. Presence of characteristic features of GCA on temporal artery biopsy in relation to clinical and ultrasound findings.

13. RESEARCH GOVERNANCE

The sponsor for the study will be the University of Oxford. We will set up a Trial Steering Committee (TSC) with an independent chair, at least two independent clinicians and at least one patient member. The TSC will meet once a year after commencement of the study to oversee the project, or as earlier if required. The Chief Investigator (Dr Raashid Luqmani, RAL) will attend to give an update on study progress and report any problems arising. An invitation will be extended to observers from the Health Technology Assessment (HTA, Funder of the Study) to attend the TSC meetings.

The study management committee will be chaired by RAL, who will take overall responsibility for the study. The investigators will be required to attend their relevant training sessions to standardize ultrasound scanning techniques and interpretation, and biopsy preparation and interpretation respectively. The study group will meet every 6 months to discuss progress of the study.

An independent Data Monitoring Committee (DMC) will meet at least once a year review the study data and safety issues.

A dedicated Trial Coordinator will be appointed (to be based in Oxford) who will be responsible for overall coordination of the study under the supervision of Dr Luqmani. Sheffield University's Clinical Trials Research Unit (Mike Bradburn) will be responsible for the management and analysis of the cohort study; Allan Wailoo (University of Sheffield) will be responsible for the cost-effectiveness study; and Andrew Hutchings (London School of Hygiene & Tropical Medicine) will be responsible for the assessment of inter-observer agreement and the expert panel component.

13.1 Project timetable and milestones

The project will be completed in four years, as shown in the following table. (* denotes pre-project activity)

	*	Year 1						Year 2						Year 3						Year 4					
		JF	MA	MJ	JA	SO	ND	JF	MA	MJ	JA	SO	ND	JF	MA	MJ	JA	SO	ND	JF	MA	MJ	JA	SO	ND
Recruit research staff																									
Establish Steering Group																									
MREC Ethics and research governance																									
Develop cohort study documentation/SOPs																									
Develop and deliver US and biopsy training																									
Approve centres for participation																									
Cohort study recruitment																									
Cohort study follow-up (inc quality control)																									
Recruitment monitoring																									
Additional centre training & recruitment if required																									
Develop web-based US and biopsy assessment																									
Inter-rater assessment of US and biopsy images																									
Develop and pilot clinical vignettes																									
Expert panel review of clinical vignettes																									
Database final cleaning & locking																									
Data analysis																									
Preparation of final report & manuscripts																									

Key milestones are shown below at 6-monthly reporting intervals starting at the end of month 6.

Date	Milestones
Month 6	M6.1 Study materials/protocols prepared; M6.2 Ethics and research governance approval complete; M6.3 Centres trained, approved and ready to recruit.
Month 12	M12.1 Recruitment monitoring report; M12.2 Quality control report.
Month 18	M18.1 Recruitment monitoring report; M18.2 Quality control report; M18.3 Additional centres (if required) trained, approved and ready to recruit;
Month 24	M24.1 Recruitment monitoring report; M24.2 Quality control report.
Month 30	M30.1 Recruitment monitoring report; M30.2 Quality control report; M30.3 Web-based US and biopsy assessment developed.
Month 36	M36.1 Recruitment completed; M36.2 Clinical vignettes (web-based) developed.
Month 42	M42.1 Follow-up completed; M42.2 Inter-rater assessment of US and biopsy images analysis competed; M42.3 Expert panel review of vignettes completed.
Month 48	M48.1 Database cleaned and locked; M48.2 Analysis completed; M48.3 Final report completed.

14. PARTICPIANT RECRUITMENT

Ensuring adequate recruitment is key to the success of this study. In preparing the full proposal we surveyed potential collaborating centres to ascertain, where possible, audit data on numbers of temporal artery biopsies for suspected GCA and a realistic estimate of likely study recruitment (based on previous recruitment to GCA and related studies where available). We plan to have 25 centres participating in the study (19 have already agreed and provided estimates of recruitment). We estimate that, based on audit data, the 25 centres will perform a total of approximately 450 temporal artery biopsies for suspected GCA per year, with realistic estimates for study recruitment of 244 per year, or 10 per centre on average. In order to achieve the target sample size of 402 we need to recruit an estimated 430 participants at a rate of 172 per year (allowing for losses to follow-up due to missed or delayed ultrasound/biopsy appointments) for 2.5 years. Even if recruitment at the 25 centres falls 30% below estimates, it will still be possible to achieve the required sample size within the 2.5 year recruitment timescale. Early monitoring of recruitment and follow-up rates will be carried out. In the event of under-recruitment, contingency arrangements are: firstly, to recruit additional centres as appropriate in England and the rest of the UK; and secondly, to recruit European centres through the applicants' links with EUVAS (subject to NIHR HTA approval).

Factors that we believe will help recruitment are: minimal additional burden to participating patients, with no alteration to usual care; reserve staff at each collaborating centre trained in each of ultrasound and biopsy, to minimize lost recruitment due to staff absence; promote study to GPs and relevant hospital departments; and, collaborating centres funded on a per-recruited participant basis (refer to Table 1).

Table 1 Estimated annual recruitment by centre

Centre	*Estimated annual recruitment based on an audit of all centres approached*
Birmingham	20
Cambridge	7
Derby	10
Dudley	5
Hammersmith/St Mary's	10
King's College	5
Leeds	25
Liverpool, Aintree	10
Manchester	10
Newcastle	10
Nottingham	10
Oxford (NOC and JR)	40
Romford (Queens Hospital)	5
Sheffield	5
Southampton	5
Southend	23
Stoke	10
9 further centres	45
Total annual estimate	**255**

14.1 Participant confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

14.2 Expenses for participants

Participants will be offered travel expenses to cover the cost of any extra journeys to hospital for the purpose of the study

15. EXPERTISE

The proposed study brings together a group of researchers and clinicians with extensive experience in conducting and participating in large collaborative studies. All key clinical specialties are represented as well as expertise in medical statistics and epidemiology (Mike Bradburn, Andrew Hutchings) and health economics (Allan Wailoo).

Raashid Luqmani has recently coordinated and completed a large clinical study of disease assessment in systemic vasculitis in the UK and is currently analysing the results of another large multicentre study of vasculitis in Europe; both have involved recruitment of >300 patients with systemic vasculitis. Dr Luqmani has a longstanding interest and expertise in systemic vasculitis, chairs the British Society for Rheumatology (BSR) Special Interest Group on Vasculitis and has participated in several multicentre trials of vasculitis. Dr Luqmani will have overall responsibility for the project and Andrew Hutchings will deputise. Bhaskar Dasgupta and Andrew Hutchings have a longstanding interest and expertise in polymyalgia rheumatica and giant cell arteritis and have produced BSR guidelines on the management of GCA. Professor Dasgupta is co-ordinating a large international study to develop diagnostic criteria in

polymyalgia rheumatica, and chairs the BSR Special Interest Group. Andrew Hutchings previously worked on a large MRC-funded study of expert panels.

Expertise in the technologies being assessed is provided for ultrasound by Wolfgang Schmidt (a world expert on ultrasound of temporal arteritis who has agreed to be a consultant to the study) and Eugene McNally, an expert radiologist with considerable ultrasound experience. Richard Wakefield is a rheumatologist with a longstanding academic interest in musculoskeletal ultrasound. Dr McDonald and Dr Wolfe are expert histopathologists who are responsible for reviewing the majority of the temporal artery biopsies in their departments.

The cohort study will be co-ordinated by the Clinical Trials Research Unit at the University of Sheffield, which is already co-ordinating several HTA-funded studies. Allan Wailoo (University of Sheffield) is an experienced Health Economist with considerable expertise in rheumatic diseases and who will supervise the cost-effectiveness study.

Other clinical specialties involved in the diagnosis and treatment of GCA are represented by Dr Hamilton (an academic general practitioner) and Mr John Salmon (ophthalmologist). An experienced study co-ordinator will be recruited and sited in Oxford with other study co-ordinators with experience of undertaking large multi centre musculoskeletal research studies.

Key collaborators to the study include many rheumatologists with an expertise in vasculitis, as well as general rheumatologists seeing patients with a variety of rheumatic conditions. Centres involved include a mix of teaching and non teaching hospitals to ensure generalisability of the findings. As well as Oxford, Leeds and Southend they include: Birmingham, which has good links to the Eye Hospital on site (David Carruthers), Cambridge (Frances Hall), Derby (Nicolas Raj), Hammersmith Hospital London (Justin Mason)., St Mary's Hospital London (Anne Kinderlerer), Ipswich (Richard Watts), Kings College Hospital (David L Scott), Newcastle (Phil Platt), Norwich (David GI Scott), Nottingham (Peter Lanyon), Poole General Hospital (Paul Thompson), Romford (Kuntal Chakravarty), St George's Hospital, London (Patrick Kiely), Southampton (Andrew Lotery), Stafford (Tom Sheeran), Stoke on Trent (Sanjeet Kamath), Sheffield (Gerry Wilson), Liverpool (Cristina Estrach), Portsmouth (Richard Hull), Aylesbury (Malgosia Magliano), Manchester (Pauline Ho).

16. DEFINITION OF END OF STUDY

For study participants, the end of study is the date of the last visit for the study of the last participant.

For the overall study, this is expected to be 6 months after the study participant end of study definition above (refer to section 13.1 for project timetable and milestones).

17. SAFETY

17.1 Definition of Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence that:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events.*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

17.2 Reporting Procedures for Adverse Events (SAE and AE)

A serious adverse event (SAE) occurring to participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was: 'related' – that is, it resulted from administration of any of the research procedures; and 'unexpected' – that is, the type of event is not part of the study as an expected occurrence. Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES report of serious adverse event form (see IRAS/NRES website).

As part of the study adverse reactions/events will be reported to the clinician or research nurse responsible for the participant's care. All forms will be sent by fax or e-mail to the trial co-ordinator in Oxford as soon as possible. These will be discussed in both Trial Steering Committee (TSC) and Data Monitoring Committee (DMC) meetings.

18. SERVICE USERS

Patients have been asked to comment on the initial protocol and offer strong support. We will be arranging for a patient representative on the data monitoring committee, and also on the trial steering committee.

19. PUBLICATION POLICIES

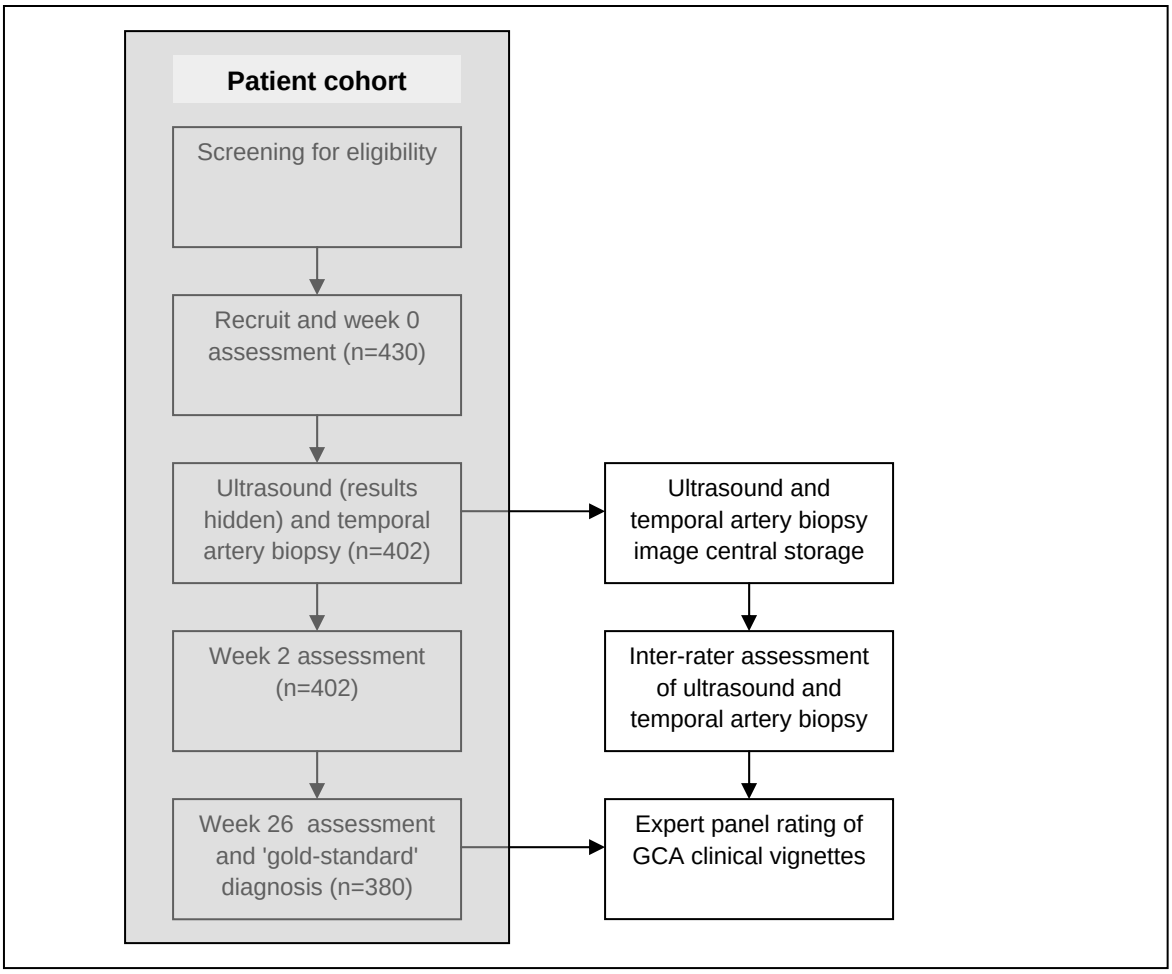
The investigator(s) will retain editorial control over manuscripts, abstracts press releases, and meeting presentations. The NIHR HTA (as funder) will be acknowledged in all publications. Authorship will be according to ICMJE recommendations (http://www.icmje.org/ethical_1author.html)

20. FINANCING AND INSURANCE

The study is funded by the National Institute for Health Research Health Technology Assessment (NIHR HTA) programme. The University of Oxford, as sponsor to the study will be responsible for obtaining insurance for the study

21. TABUL FLOW DIAGRAM (PRIMARY RESEARCH ONLY)

Patient recruitment and follow up with data collection



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23. APPENDICES

23.1 Appendix I Substudies and creation of a biobank and teaching tool

As a part of this proposal we plan to create a biobank of temporal artery biopsies and accompanying serum, plasma and peripheral blood cells from participating study subjects who have suspected or confirmed diagnosis of GCA. In addition we plan a number of sub-studies, subject to further funding. This will form Part B of the Consent/Assent form. If the potential participant agrees to participate only in the main body of this research protocol and is willing to provided fully written informed consent or assent they will be asked to only sign Part A of the Consent/Assent form and their blood samples and human tissue will not be stored for the purpose of any research. If the potential participant agrees for the analysis and storage of their temporal artery biopsy, serum/plasma and peripheral blood cells for this research study and any related studies which may occur in the future they will be asked to complete Parts A and B of the Consent/Assent form. It will be made very clear in the Consent/Assent and Participant/Patient Information Sheet (PIS) the additional consenting to the sub studies and/or Biobank part of this study. Potential participants will be unable to participate in Part B of the study if they are unwilling to consent to Part A of the study. We plan to obtain and store the participant's temporal artery biopsy between their enrollment visit (Visit 1) and day 7. We plan to obtain and store 85 ml blood (EDTA whole blood [at Study Visit 1 only] and plasma and serum samples) in total for all 3 study visits. The current role of biomarkers is uncertain in diagnosis of GCA; CRP is of limited value as is the ESR. Measurement of serum cytokines is feasible but failed to discriminate patients with GCA from other conditions. It would be interesting to examine the role of a gene expression profiling to the diagnosis of GCA and the clinical study proposed lends itself well to a series of biomarker studies. The use of peripheral blood mononuclear cells for genomic analysis is becoming more widely available. The large collection of images of pathological features of GCA linked to clinical findings and ultrasound images is ideal for the development of a teaching website. We plan collaborative projects with:

(i) Dr David Greaves (Reader in Pathology at the Sir William Dunn School of Pathology, Oxford) who has a long-standing interest in macrophage and chemokine biology in the context of inflammation. In work funded by the British Heart Foundation, the MRC and the Wellcome Trust, Dr Greaves has validated a panel of monoclonal antibodies (mAb) that detect macrophage-specific antigens (CD68, and SR-A) as well as mAbs that detect specific sub-sets of macrophages (MMR and Dectin) and chemokines (MCP1, MDC and fractalkine). The laboratory has experience of measuring cytokine levels by ELISA and Luminex technologies using plasma samples from patient and control cohorts collected at a number of centres. This expertise is complemented by a range of techniques to assess chemokine bioactivity including the use of modified Boyden chamber assays, Ca2+ flux assays and recently the use of beta-arrestin signalling assays using the DiscoveRx system. In collaboration with other members of the cardiovascular research community Dr Greaves is well placed to initiate gene association studies using markers for genes known to be important in inflammation and macrophage biology e.g. MMP-2 . Finally, through collaboration with Dr Aron Chakera (Nephrology) and Dr Robin Choudhury (Cardiovascular Medicine) the Greaves laboratory is setting up assays of T cell and monocyte function using human whole blood samples.

(ii) Dr Ann Morgan (HEFCE Clinical Senior Lecturer Biomedical Research Unit, Leeds) runs the molecular rheumatology laboratory in Leeds. There is no validated or generally-agreed scoring system for the histopathological features of GCA that has been adopted into routine clinical practice. A large retrospective review of pathological reports from multiple centres has recently suggested that giant cells, neoangiogenesis, intimal thickening, arterial occlusion and possibly plasma cells may predict visual complications in GCA. Semi-quantitative measures of inflammation and giant cells developed to date appear to be poorly reproducible between different observers, with only assessment of the degree of intimal hyperplasia/arterial occlusion demonstrating good inter-observer agreement. This was found to correlate with neuro-ophthalmic symptoms in GCA. An objective scoring system that records a greater number of facets of disease pathogenesis, including inflammation, may be a more powerful tool in clinical practice,

allowing the histological features of disease to be compared between sites in multicentre research studies and audit. Dr Ann Morgan and Dr Sarah Mackie in conjunction with Dr Aruna Chakrabarty, a consultant histopathologist in Leeds, are currently developing objective scoring systems for GCA using a large cohort of GCA patients recruited for immunogenetic, soluble and tissue biomarker studies. The utility of computerized image analysis and special stains, for example van Gieson stains for elastin or immunohistochemical stains for characterizing the cellular infiltrate are being evaluated as diagnostic and prognostic biomarkers. In addition, any novel biomarkers that are identified by this group will be validated in the current study.

(iii) Dr Nick Platt (University of Oxford, Weatherall Institute of Molecular Medicine) will assess the extent of inflammasome activation in blood cells and tissue cells from patients with GCA. This will include characterising the nature of the cells and expression of NALP 1, 2 and 3, CASPASE 1, 3, 5 and 11, IL1 beta. In addition we will measure the degree of apoptosis and proptosis in circulating cells and tissue cells derived cells in patients with giant cell arteritis. We will assess the presence and functional characteristics of suppressor phenotypes (including T regulatory cells and myeloid suppressor cells). We will perform a FACS analysis for these populations, as well as tissue histology and immunostaining of sections. We will determine the functional effect of these cells by measuring suppression of T cell proliferation.

(iv) Professor David Dewhurst (University of Edinburgh, Learning Technology Unit) who has significant expertise in e-learning. The creation of a large databank of ultrasound images, pathology slides and the clinical features of a cohort of patients with suspected GCA forms a useful teaching tool; we propose to develop this as a web based teaching tool to assist in the training of the use of ultrasound technology, correct interpretation of histology, as well as a teaching tool to understand more about the clinical presentation of giant cell arteritis. The creation of a searchable, well-catalogued online repository of learning objects will promote sharing and reusability by providing teachers (as well as undergraduates, and trainees) access to quality-assured resources which they can readily download and incorporate into their teaching and learning.