NHS
Health Research Authority

FULL/LONG TITLE OF THE STUDY

PROSPECT-PLEURA: Biomarker Discovery to Refine Diagnosis and Prognosis in Histologically Indeterminate Pleural Effusions

SHORT STUDY TITLE / ACRONYM

PROSPECT-PLEURA

PROTOCOL VERSION NUMBER AND DATE

- 1.0 20/04/2026

RESEARCH REFERENCE NUMBERS

IRAS Number: 366402

SPONSORS Number: -

FUNDERS Number: RCCPDB-Nov25/100012

CPMS Number: 72537

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:

..../...../.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Signature: Date:

..../...../.....

Name: (please print):

.....

LIST of CONTENTS

KEY STUDY CONTACTS

Chief Investigator	Dr Lucy Jackson-Jones, Senior Lecturer, Faculty of Health and Medicine, Lancaster University l.jackson-jones@lancaster.ac.uk B006, Furness Building, Lancaster University LA1 4YW
Principal Investigator - UHMBT	Dr Timothy Gatheral, Respiratory Medicine Consultant, University Hospitals of Morecambe Bay NHS Foundation Trust Timothy.gatheral@mbht.nhs.uk Royal Lancaster Infirmary, Ashton Road, Lancaster LA1 4RP
Co-Principal Investigator - UHMBT	Ms Gemma Barton, Pleural Nurse Consultant, University Hospitals of Morecambe Bay NHS Foundation Trust Gemma.barton@mbht.nhs.uk Royal Lancaster Infirmary, Ashton Road, Lancaster LA1 4RP
Co-Investigator	Dr Oliver Mann, NIHR Academic Clinical Fellow, Faculty of Health and Medicine, Lancaster University ST1 Specialty Trainee in Respiratory Medicine, University Hospitals of Morecambe Bay NHS Foundation Trust O.mann@lancaster.ac.uk Oliver.mann@mbht.nhs.uk
Study Management Group	Dr Lucy Jackson-Jones, Dr Oliver Mann, Dr Timothy Gatheral, Ms Gemma Barton
Sponsor	Lancaster University Designated representative: Becky Gordon, Head of Research Policy and Policy, Lancaster University, LA1 4YT sponsorship@lancaster.ac.uk
Funder(s)	Cancer Research UK
Key Protocol Contributors	Dr Lucy Jackson-Jones, Dr Oliver Mann
Committees	

STUDY SUMMARY

Study Title	PROSPECT-PLEURA: Biomarker Discovery to Refine Diagnosis and Prognosis in Histologically Indeterminate Pleural Effusions
Internal ref. no. (or short title)	PROSPECT-PLEURA
Study Design	Diagnostic test accuracy study and prospective observational clinical trial.

Study Participants	Adult NHS patients undergoing thoracocentesis, chest drain or indwelling pleural catheter insertion for unexplained pleural effusion.
Planned Size of Sample (if applicable)	150 participants 30 participants who have donated two pleural effusion samples, meaning a total of 180 pleural effusion samples from 150 participants
Planned follow-up period	3 years
Planned Study Period	1 year
Research Question/Aim(s)	Can biomarkers that predict clinical outcome in atypical mesothelial proliferation and other indeterminate pleural sample histology results be identified?

STUDY SPONSOR AND FUNDER

The study sponsor will be Lancaster University.

The first year of this study has been funded by a Cancer Research UK (CRUK) Predoctoral Bursary Award held by Dr Oliver Mann (co-investigator). Dr Oliver Mann is also in receipt of a National Institute of Health and Care Research (NIHR) academic clinical fellowship award.

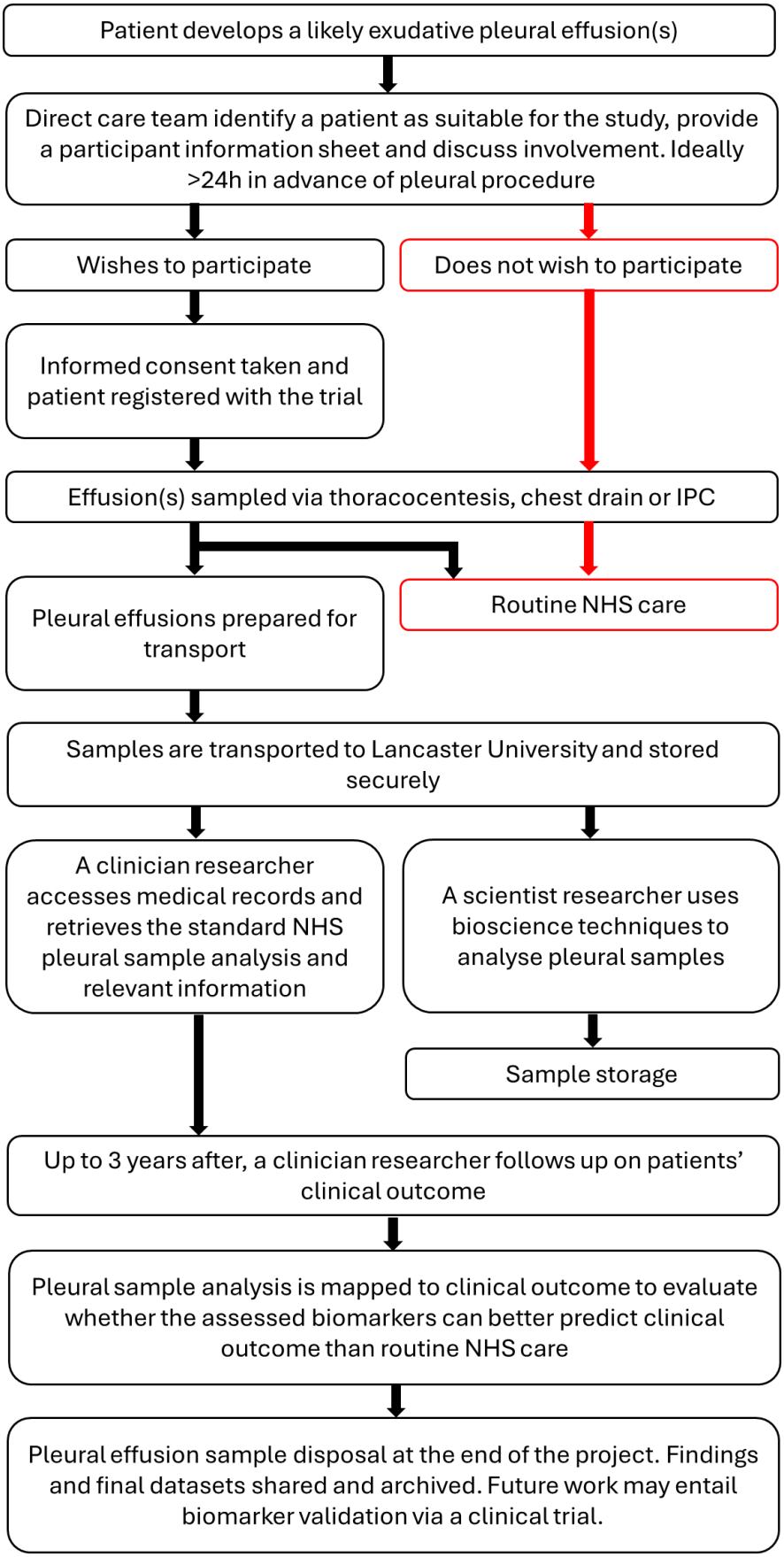
PROTOCOL CONTRIBUTORS

The protocol was designed by the chief investigator, co-investigator, principal investigator and co-principal investigator.

The NIHR and CRUK had no input on the protocol.

Lancaster University reviewed the protocol as part of the process of agreeing to sponsorship.

STUDY FLOW CHART



PROSPECT-PLEURA: Biomarker Discovery to Refine Diagnosis and Prognosis in Histologically Indeterminate Pleural Effusions

STUDY PROTOCOL

1 BACKGROUND and RATIONALE

Pleural effusion is the pathological collection of fluid between the linings of the lung and thoracic wall. An array of more than 60, sometimes concomitant, causes underpin the formation of pleural effusions ranging from temporary post-infectious changes to terminal malignant cancers (1, 2). Determining the cause of an effusion is an important clinical step with significant implications for patient management.

Currently, diagnostic evaluation of pleural fluid obtained via thoracocentesis relies on limited methodologies, and an estimated 30% of pleural effusions cannot be categorised via the current diagnostic approach (3). First, evaluating samples biochemically according to 'Light's criteria' (4), or similar, can help filter out effusions caused by oncotic or hydrostatic pressure changes. Microbiological culturing and pH analysis of samples can further assess for pleural infection, but if cytological analysis is inconclusive difficulties arise in distinguishing between several remaining causes.

Highly invasive procedures such as thoracoscopy and pleural biopsy can be considered in the hope of obtaining high-quality samples with richer cell content, but many patients are not suitable for these due to frailty or co-morbidities, and even thoracoscopy cannot offer certainty (5). Pleural histopathology and cytology requires specialist expertise not available to all healthcare services and a labour-intensive process with multiple ancillary techniques required (6), often leading to months-long processing times. High-risk malignant pleural effusions have a median survival time of just 44 days after diagnosis (7), putting this diagnostic delay into context. Prompt diagnosis of pleural effusions, ideally with less invasive procedures such as thoracocentesis, is paramount to improving outcomes of primary and secondary pleural cancers (8).

The pleura poses a particular challenge to diagnosis via cytology, as significant morphological overlap between the appearance of benign reactions and cancerous states occurs; especially true for the mesothelium (6, 9, 10). As such, histologists may report samples using terminology such as non-specific pleuritis, or atypical mesothelial proliferation in the case of the mesothelium (11). In areas of low tuberculosis prevalence the default assumption is that pleural inflammation is 'malignancy until proven otherwise' (3), but malignancies may sometimes take years to fully manifest.

Overall sensitivity of pleural fluid cytology was estimated at 46% in the UK (12) and 58% in a recent meta-analysis (13), approximately equal to a coin flip. There is, however, broad variation in sensitivity between different primary cancer types, reaching as low as 6% in malignant pleural mesothelioma (MPM) and up to 95% in ovarian cancer (12).

This overall diagnostic uncertainty regarding malignant pleural effusion poses significant challenges for clinicians and patients, but earlier diagnosis may shift stage distribution and allow more patients access to curative-intent therapies.

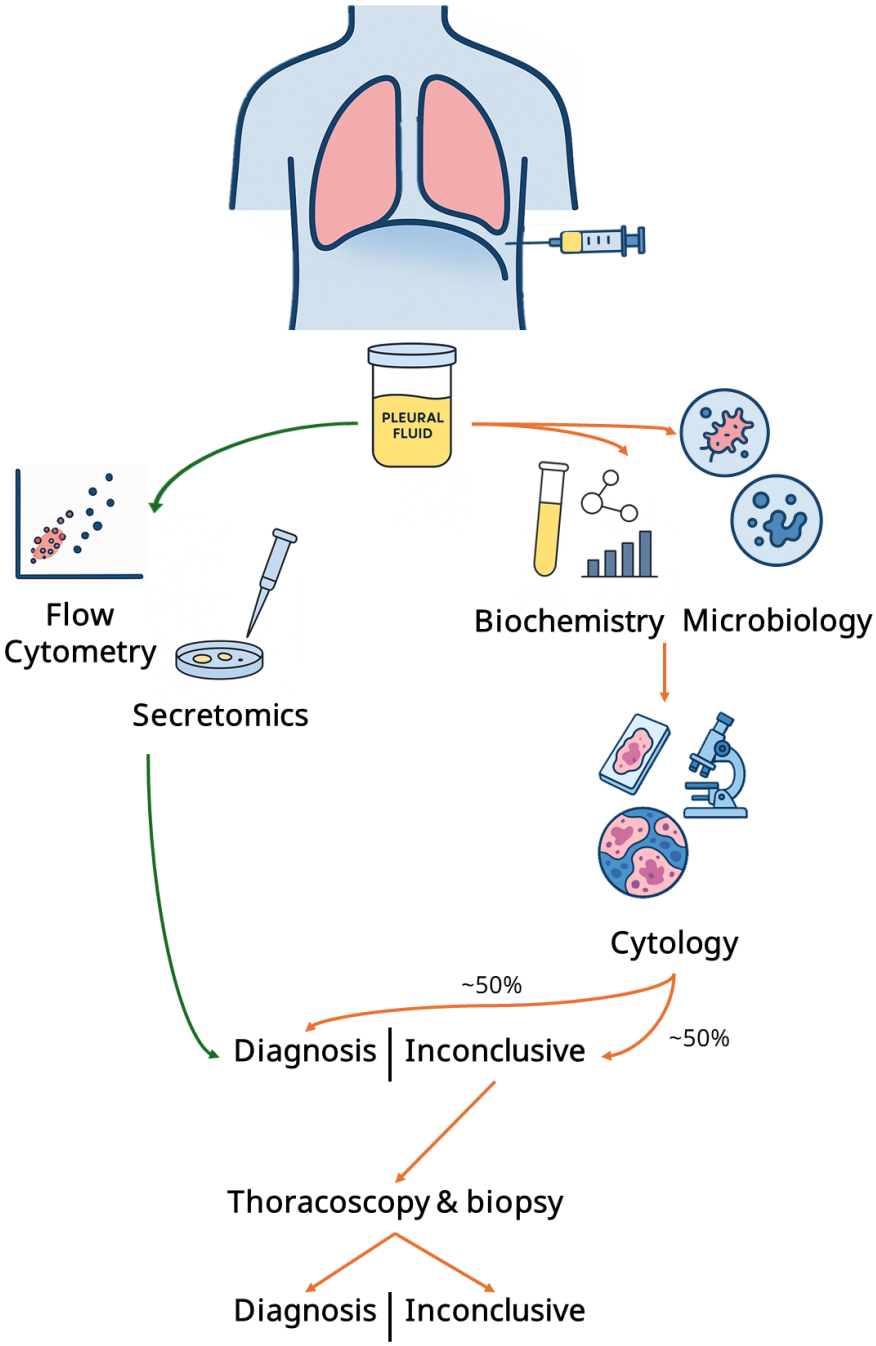
Many biomarkers have been reported that try to triage malignant from benign pleural effusions or diagnose between subtypes of malignant conditions. These span nucleotides and genetics (14, 15), immunohistochemistry staining (9), metabolites (16), and proteins (17) which have been reviewed here (8, 18, 19). Few of these purported biomarkers have been externally validated even pre-clinically (8, 20), and when several metabolites were assessed (21) poor reproducibility highlighted the pressing need to do this. Furthermore, most studies are limited to proving 'correlation' alone and few investigate the underlying 'causation' mechanism that may link the pleural biology to the biomarker. Clear causality mechanistic insight supporting a biomarker will maximise the chance that its presence represents genuine malignancy, and not a simply detecting a molecular abnormality, as can befall BAP1 and MTAP in MPM and mesothelioma in situ (22).

One promising candidate biomarker for pleural fluid requiring external validation and deeper pleural pathophysiology understanding is hyaluronic acid (HA) and its principal receptor CD44. As reviewed by Cortes-Dericks and Schmid (23), multiple clinical trials have confirmed an association between MPM and elevated HA concentrations, CD44 expression, or both in pleural fluid or biopsy samples. However, virtually all mechanistic insights to HA/CD44 biology in mesothelial malignancy are derived from immortalised tumour cell lines (24), with only a handful originating from pleural biopsy samples (25, 26). Greater exploration of the role of HA/CD44 signalling in primary human samples is required. Using patient pleural fluid samples is less invasive and more readily available than biopsy samples, whilst still reflective of adult human lungs which have experienced and adapted to a lifetime of exposures from infection to inhaled irritants.

Macrophages, which express CD44 and other HA receptors, are key regulators of tumour progression and have been shown to have close associations with mesothelial cells in pre-clinical models (27, 28). Detailed immune characterisation of human pleural fluid is limited, with few in depth studies characterising the myeloid compartment (29, 30). Preliminary data from the Jackson-Jones laboratory reveals multiple myeloid populations and the presence of free-floating mesothelial cells within pleural fluid of patients with diverse causes of effusion. Advanced understanding of the relationship between mesothelial cells and macrophages will be essential in enhancing knowledge, and later manipulation, of tumour pathogenesis. The response of macrophages or other pleural immune cells during malignancy or infection may further provide a source of potential biomarkers that can differentiate effusion causes.

More broadly, the architecture of the pleura on a molecular and cellular level is still being characterised. Application of multi-omics and next-generation sequencing in pleural pathology is behind other fields (31), but when applied offered critical insights into the functions of pleural immune cells (32, 33) and stromal cells (34).

2 THEORETICAL FRAMEWORK



3 RESEARCH AIMS

Our research aims to detect molecular and cellular characteristics that can differentiate histologically indeterminate pleural samples into their various causes. Of particular focus for this research are pleural aspirations which are described by histopathologists as ‘atypical mesothelial proliferation’.

3.1 Primary Objective

- Refine the diagnostic value of pleural samples interpreted cytologically as ‘atypical mesothelial proliferation’

3.2 Secondary Objectives

- Refine the diagnostic value of pleural samples in other categories of non-specific cytology and histology.
- Triage pleural samples according to likelihood of malignancy
- Refine the prognostic yield of pleural fluid samples
- Validate previously reported pleural biomarkers that attempt to do the above.
- Assess biomarker diagnostic time compared to current NHS diagnostic pathways
- Enhance understanding of pleural pathophysiology, via characterisation of cellular populations through nucleic acid sequencing, flow cytometry, secretome profiling and microenvironment analysis, and via metabolomics of pleural fluid and cellular content.

3.3 Outcomes

Primary Outcome

- A final clinical diagnosis, cytology report(s) and biomarker analyses. Final clinical diagnosis is the diagnosis for the formation of a pleural effusion, as determined by the clinical care team. Final diagnosis can be made posthumously.

Secondary Outcomes

- Additional information on clinical outcomes, including:
    a. Pleural fluid biochemistry and microbiology reports
    b. Pleural biopsy reports
    c. Time to diagnosis
    d. Survival time
    e. Treatment(s) and response
- Insights on pleural pathophysiology at the molecular and cellular level

4 STUDY DESIGN and METHODS of DATA COLLECTION

4.1 Study design

The design of the research is a diagnostic test accuracy study and prospective observational clinical trial. The study protocol will be registered with the ISCRTN prior to research commencing.

4.2 Study Population

The study aims to collect pleural fluid samples from a broad range of causes to represent the cohort of patients presenting to a pleural clinic for diagnosis of a suspected exudative pleural effusion.

The following eligibility criteria will be uniformly applied to all participants.

4.2.1 Inclusion criteria

- Undergoing medically necessary pleural fluid sampling or removal, via any method.
  AND
- The pleural effusion is suspected or known to be exudative.
  AND
- Aged 18 or older and has capacity to consent to participate.

4.2.2 Exclusion criteria

- Latent, active, or suspected tuberculosis, whether pulmonary or extra-pulmonary.

- Clinically unstable patients requiring emergency removal of pleural fluid.
- Previous pleurodesis.
- Participating in ongoing PROSPECT-PLEURA patient and public involvement.

4.2.3 Justification of eligibility criteria

To allow nomination of new biomarkers, we plan to conduct single-cell RNA sequencing on pleural effusion samples to interrogate gene expression and signalling pathways within cellular content and pair this with analysis of cellular activation and bio-chemical analysis of secreted factors released by cells and within the pleural fluid. RNA sequencing is a resource-intense molecular technique and as such there are limits on how many samples can be processed. To draw conclusions, the clinical outcome of the patient who has donated the sample will need to be known. As it can take months for the cause of an effusion to become apparent via current diagnostic pathways, and samples may need to be fixed and processed before clinical outcome is known, to safeguard limited resources we will require some samples from patients with an existing diagnosis. The inclusion criteria are broad to allow for this, for example surplus pleural fluid from patients who are re-presenting for drainage of a recurrent effusion or patients presenting for regular removal of fluid from an indwelling pleural catheter.

The criteria are also broad to allow a large range of causes to be subject to later biomarker validation which will provide a sufficient variety of control samples and improve translatability of any findings.

Tuberculosis is listed as an exclusion criterion to minimise the risk of infection to researchers handling pleural fluid samples. Pleurodesis may alter the immunological landscape of the pleura and increases the difficulty of obtaining pleural samples from the affected hemithorax.

4.3 Participant Identification and Informed Consent

The study will be single site. Patients will be identified for recruitment to the study by a member of the direct care team at University Hospitals of Morecambe Bay NHS Foundation Trust (UHMBT), using the eligibility criteria above. Members of the direct care team will keep a Screening Log record of all potential participants screened for inclusion. UHMBT research staff or the clinical care team with appropriate permissions will provide a participant information sheet to patients and discuss involvement, ideally more than 24h before sample collection to allow participants time to consider involvement. In some circumstances, consent, registration and sample collection may be completed on the same day due to the single visit nature of the study. If completed on the same day participants will be given sufficient time to consider participation and must not be pressured into deciding. Their clinical care must not be compromised to facilitate participation in the study, for example delaying urgently needed intercostal drain insertion to allow time to consider involvement.

A Good Clinical Practice-trained member of the research team or UHMBT research staff with appropriate permissions will then take patient consent.

The consent process will include:

- Discussion between the potential participant and an individual knowledgeable about the research, about the nature and objectives of the study and possible risks associated with their participation.
- The presentation of written material, such as the Participant Information Sheet
- The opportunity for potential participants to ask questions.
- Assessment of capacity, as per the Mental Capacity Act (2005).
- Informing the participant of the option to withdraw consent at any time, without providing a reason.

An Informed Consent Form will be initialled and signed by the participant and signed by the staff member taking consent. Digital and physical copies will be made. The participant, medical records, site file and Lancaster University will all receive a copy. Original physical forms will be retained at

UHMBT research offices in a locked site file room. Participants will be made aware that their clinical data may be accessed and used even if they have deceased, within the 3-year timeframe.

4.3.1 Eligibility to Take Consent of a Participant

For a researcher team member with appropriate permissions or UHMBT research staff to be eligible to take a patient's consent, they must:

- Understand the study's nature, objectives and activities.
- Have a knowledge of and experience applying the principles of the Mental Capacity Act (2005) to determine if a patient has capacity to consent to participate.
- Possess an in date Good Clinical Practice certificate, which will be recorded by the principal investigator, co-principal investigator or their nominated delegate and stored electronically.
- Provide a research CV to the principal investigator, co-principal investigator or their nominated delegate demonstrating suitability and experience to act as a researcher on the study.
- Be a member of the direct care team, or have verbal consent from the patient to approach them in order to take consent.

4.4 Registration

After recruitment, participants details will be recorded on a Participant Identification Log (PIL). Ideally registration on the PIL would occur before sample collection. However, to minimise disruption to NHS services where the samples are collected (e.g. pleural clinics), pleural fluid collection for research may begin after consent forms have been signed. Nevertheless, samples must not be transported from UHMBT premises until the participant is registered on the PIL.

4.5 Pleural Effusion Sampling

The clinical care team or researchers with existing training will collect pleural fluid samples using standard thoracentesis, intercostal drain or indwelling pleural catheter (IPC) procedures. Procedures are separated into two categories:

1. Therapeutic thoracocentesis, intercostal drain and IPC. These procedures will be performed unaltered, collecting pleural effusate which would normally become clinical waste. Up to 200mL of pleural fluid may be collected for research purposes.
2. Diagnostic thoracocentesis. The procedure will be performed as standard, with the first portion of pleural fluid collected for and processed as per NHS standard pathways. Up to 50mL of residual fluid beyond clinical requirements will then be collected for research purposes. If a deterioration in clinical condition occurs during the section of a diagnostic thoracocentesis where pleural samples are being collected for research purposes, the clinician taking the sample will stop immediately and treat them as they would normally do so.

All research pleural fluid samples, regardless of thoracic procedure, will be stored in a sterile falcon tube and clearly labelled as 'for research purposes only', along with a randomly generated study ID and the date of collection.

Research samples will be handled with appropriate PPE to mitigate the small risk of transmission from a pleural sample relating to an infectious cause. Samples will be stored on ice immediately after collection until they can be transported to Lancaster University.

Before transport to Lancaster University, samples will be checked to ensure clinical identifiers such as name, NHS number and hospital number have not been added, or if added removed. Samples will be assigned a randomly generated study ID and be dated. The PIL will be contain both real-world identifiers and the study ID, acting as a cipher. The PIL will be stored securely on UHMBT IT systems and only clinical researchers or UHMBT research staff will have access to it. A double-entries logbook will be maintained at each UHMBT site participating in the research. A first entry will occur to record all

samples that are collected and stored. A second entry will be made when samples are transported to Lancaster University, stating the time, date and person who transported the sample.

4.6 Transport

Samples will then be transported to Lancaster University on the same day as collection on ice in compliance with HTA Code E, T1 traceability requirements for relevant material.

On arrival at Lancaster University, another double-entries logbook of samples will be kept recording arrival time, date and who transported the sample. Missing samples will be reported immediately to the study management group.

4.7 Sample Processing

Samples will be centrifuged and divided into supernatant and cell pellet. Supernatants will be frozen at -80ºC, and cell pellets will be fixed using Transfix® or otherwise preserved. Sample components will be stored securely in a laboratory on a research floor that can only be accessed by authorised users via a university-managed access control system. Frozen samples will be stored within a dedicated keypad locked room on the same research floor.

Some samples will not be fixed and instead cultured in containment level 2 culturing facilities at Lancaster University and disposed according to standard operating procedures after analysis. *In vitro* cell lines will not be established from these samples.

4.8 Scientific Analysis

Members of the research team will probe the molecular and cellular aspects of the collected pleural fluid. The precise scientific analysis to be completed, especially in regard to better understanding pleural pathophysiology, may evolve as the study progresses and will be responsive to the direction the chief investigator wishes to pursue. At present, several analyses are envisaged. First, we will conduct single-cell RNA sequencing on cellular content from pleural fluid to resolve the transcriptional states of immune and mesothelial cells present within pleural fluid, among others. We will apply this to samples from known outcomes where possible, (e.g. lung adenocarcinoma, pleural infection, breast carcinoma), and compare these to indeterminate mesothelial proliferation or non-specific pleuritis. The resulting insight into how the pleural tumour microenvironment is shaped will allow nomination of novel biomarkers. In addition to generating new data, we will also attempt to mine publicly available sequencing data sets that would be appropriate for our objectives.

We will then use multiplex flow cytometry to assess immune and mesothelial cell populations present within pleural fluid and their expression of extracellular matrix ligands and receptors including CD44, Lyve1 and Icam1. Additionally, supernatant will be analysed using ELISA and multiplex array to investigate what is being secreted by immune and mesothelial cells and accumulating in pleural fluid, including hyaluronan, mesothelin and other tumour-associated molecules such as cell surface receptors, cytokines, metabolites and chemokines. Patient immune and mesothelial cells from non-fixed samples will be separated by fluorescence activated cell sorting and placed in culture. Cultured cells will be exposed to immune stimulatory agents or modulators of extracellular matrix, including within organ-on-a-chip technologies, and analysed by a variety of methods. Currently envisaged methods include: microscopy, flow cytometry and biochemical techniques including nucleic acid sequencing and ELISA to determine cellular phenotype, cell to cell interactions and individual cell type secretome.

Flow cytometry and ELISA will be used to determine the presence or concentration of cellular and supernatant biomarkers, respectively.

4.8.1 Minimising Bias

When assessing samples the scientific team will be blinded from knowing clinical outcome. This will be achieved by assigning samples a randomly generated study ID and removing clinical identifiers such as NHS number, as formerly mentioned. The usual time delay in reaching a final diagnosis detailed earlier will also help to blind scientific researchers. If clinical outcome is known to a member for the

research team involved in both the clinical and scientific elements of the study by time of processing, samples will be randomly assigned to different batches to prevent batch bias.

Clinicians will be blinded from knowing the results of scientific analyses but not having access to laboratory analysis results, which will be held on separate, accessed controlled cloud storage system.

4.9 Sample Handling at End of Project

At the end of the research project, samples will either be disposed of, transferred to a HTA licensed tissue storage facility, or re-used for another research project at Lancaster University which has gained a favourable opinion from an NHS ethics committee.

If disposed of, samples will be disposed of at the end of the research project, or earlier, following university standard operating procedures and Human Tissue Authority guidance.

The Lancaster University double-entries logbook of samples will be updated to record by whom and when samples were disposed of, or when and where they were transferred to. Missing samples will be reported immediately to the study management group.

4.10 Clinical Outcomes Data Collection

Clinical outcomes data of participants will be collected by members of the research team or UHMBT research staff with permissions from UHMBT electronic patient records. A case report form will be used to standardise data collection. Only information on the case report form will be collected. Clinical outcomes data may potentially be gathered on multiple occasions, up to 3 years after recruitment to the study. Collected data will include:

- Patient's name
- Two patient identifiers, NHS number and hospital number.
- Demographic details such as date of birth age at time of sampling, gender and ethnicity.
- Relevant blood, microbiology, biochemistry and radiology test results up to 1 year before pleural sampling
- Relevant medications, such as antibiotics, up to 2 weeks before pleural sampling
- Pleural effusion cytology reports, up to 1 year before pleural sampling
- Pleural fluid analysis, including pH, protein concentration gradients, cell counts and microbiology reports
- Pleural biopsy reports, if available
- Serum circulating tumour DNA (ctDNA) reports, if available
- The results of investigations, such as chest radiographs, CT imaging, ultrasound imaging, bronchoscopy, endobronchial ultrasound, broncho-alveolar lavage or bronchial washings, and other relevant investigations
- Clinical outcomes, such as final diagnosis, survival time, treatment, response to treatment and other relevant clinical information. Clinical outcome will be assessed by review of clinical notes, lung cancer MDT notes, oncology notes and medical or surgical treatments given
- Date of death
- The time from pleural sampling to diagnosis, including time to cytology and biopsy results

If a participant becomes deceased after sample collection, their clinical data will be accessed and used, compliant with the 3-year timeframe outlined above. The trial will not include post-mortem samples.

4.11 Data Transfer

Clinical data will be securely exported from UHMBT electronically via encrypted OneDrive links and stored at Lancaster University and linked to the sample's scientific analysis.

5 STUDY ACTIVITIES

Study activities will be the same regardless of pleural fluid sampling technique.

5.1 Visit 1

Visit 1 activities should be completed at least 1 day after provision of the PIS to allow time to consider participation, but can be completed on the same day if necessary. If completed on the same day participants must be given sufficient time to consider participation, must not be pressured into deciding, and must have full opportunity to ask questions and be answered satisfactorily. Their clinical care must not be compromised to facilitate participation in the study.

Activities

- Review eligibility criteria.
- Discussion and capacity assessment.
- Informed written consent.
- Register the patient on the participant identifier log (PIL). To minimise disruption to NHS services, pleural fluid collection for research may begin after a consent form has been signed but before registration. However, samples must not be transported from UHMBT until the participant is registered on the PIL.
- Record trial ID on consent form.
- Sample pleural fluid, via any method. The method of collection will be determined by the clinical team and must not be influenced by the study. For diagnostic thoracocentesis, after all clinically necessary samples have been obtained up to 50mL can collected for research. For therapeutic thoracocentesis, intercostal drain and IPC-derived samples up to 200mL can be collected at any time.
- Label samples with study ID and date of collection.

Study pleural fluid samples require immediate storage on ice or at 4°C.

- Record sample in double-entries logbook and store until transport.
- Record adverse events.

There are no further mandatory study visits. Collection of clinical data will occur later and does not require participant involvement.

5.2 Visit 2

Should a participant re-present for pleural fluid aspiration or removal at any future point, they will be reminded of their participation in the study and be offered the opportunity to donate any further surplus pleural fluid.

Activities

- Review eligibility criteria.
- Remind participant of previous involvement with the study.
- Remind participant of their ability to withdraw at any point without their rights being affected.
- Re-confirm consent verbally and record verbal discussion in the medical notes.
- Sample pleural fluid, via any method. The method of collection will be determined by the clinical team and must not be influenced by the study. For diagnostic thoracocentesis, after all clinically necessary samples have been obtained up to 50mL can collected for research. For therapeutic thoracocentesis, intercostal drain and IPC-derived samples up to 200mL can be collected at any time.
- Label samples with study ID and date of collection.

Study pleural fluid samples require immediate storage on ice or at 4°C.

- Record sample in double-entries logbook and store until transport.
- Record adverse events.

Participants will not be asked to provide more than two pleural fluid samples.

6 SAFETY REPORTING

6.1 Risk to participants

The study involves no additional invasive procedures. To be included in the study, participants must already be undergoing medically necessary pleural sampling.

We envisage the majority of participants will have their pleural effusion sampled using therapeutic thoracocentesis, intercostal drain or IPC techniques. For this subset of participants, all pleural fluid present in the thorax is typically drained off for symptom relief and subsequently discarded as clinical waste. This research project will convert that clinical waste into research samples. As such, there will be no additional risks to participants.

For pleural effusion sampled via diagnostic thoracocentesis, involvement in the study will prolong the time that collecting instrumentation is within the thorax by a few minutes whilst up to 50mL of additional pleural fluid is collected for research purposes. Neither the extended instrumentation time nor the removal of up to 50mL of pleural fluid are thought to pose any significant additional risks to patients.

In the very unlikely event of a deterioration in clinical condition occurring during the section of a diagnostic thoracocentesis procedure where pleural samples are being collected for research purposes, the clinician taking the sample will stop and treat them as they normally would for thoracocentesis complications. The chance of clinical deterioration is unlikely to be significantly increased by participating in this research.

6.2 Definitions

An adverse event (AE) is any untoward medical occurrence in a study participant, which is not necessarily caused by inclusion in the research

A related adverse event (RAE) is any AE which is thought to be caused by or related to the study participation.

A serious adverse event (SAE) is defined as any untoward medical occurrence in a study participant that is life-threatening or results in death, hospitalisation or prolonging of hospitalisation for the SAE, or persistent impairment/disability.

The principal investigator (or chief investigator, where appropriate) is responsible for determining the severity of any adverse event if there is ambiguity.

6.3 Event reporting

It is anticipated that very few AEs will be classified as RAEs for the reasoning outlined in section 8.1.

Expected events of pleural fluid sampling via thoracocentesis, intercostal drain or indwelling pleural catheter are similar regardless of procedure, and include:

- Pain or discomfort
- Bleeding
- Infection
- Procedure failure, dislodgement
- Drain or catheter blockage
- Surgical emphysema
- Damage to surrounding structures, such as the costal neurovascular bundle, lung, heart, diaphragm, liver or spleen
- Re-expansion pulmonary oedema
- Pneumothorax

Any AEs that occur will be recorded by UHMBT research staff in a log which will be reviewed yearly by the principal investigator or co-principal investigator to detect common trends. They will also be reported to the sponsor upon request.

6.4 Serious adverse events (SAE)

Any SAEs will immediately be reported to the chief investigator and the sponsor, following Lancaster University Governance team processes. They will similarly be recorded by UHMBT research staff and the principal investigator informed immediately.

7 STATISTICS

7.1 Population Size

A recent retrospective study at a UK tertiary centre in Oxford (35) found that 40% of exudative effusions were malignant pleural effusions. Given this high pre-test probability of malignancy, sensitivity has been prioritised as the primary accuracy parameter for sample size determination as the intended clinical role is cancer exclusion in a high-risk population. Specificity will also be evaluated to ensure downstream investigation burden from positive results remains clinically acceptable.

7.1.1 Triage Test *A Priori* Population Size Estimates

One potential output of this research is the development of a ‘triage’ test that can differentiate malignant from non-malignant causes of pleural effusions, without providing a specific diagnosis.

Pleural fluid cytology has an estimated average sensitivity of approximately 50% (12). A new triage biomarker would require a clinically meaningful improvement in sensitivity to justify adoption; an absolute sensitivity of approximately 70% has therefore been selected as the minimum target threshold for clinical utility.

Sensitivities will be compared using McNemar’s test for paired binary outcomes among cancer-positive patients. Assuming:

- 20% absolute difference in sensitivity
- Discordant proportion of 30% between tests among cancer-positive participants
- 80% power
- Two-sided alpha significance level of 5%, consistent with estimation via confidence intervals.

An estimated 60 cancer-positive participants are required. Assuming a malignant pleural effusion prevalence of 40% (35), the total sample size required is approximately 150 participants.

7.1.2 Diagnostic Test *A Priori* Population Size Estimates

In addition to triage applications, this research may develop or validate biomarkers capable of diagnosing specific pleural malignancies. Of particular interest is MPM, an aggressive malignancy that is difficult to diagnose from pleural fluid cytology, with an estimated sensitivity as low as 6% (12). There is a compelling local need to improve outcomes in MPM, as Health and Safety Executive data for Westmorland and Furness, included in the catchment area of UHMBT, has a standardised mortality rate of between 200-500 for MPM (36) reflecting a disproportionately high disease burden.

As discussed previously, hyaluronan is a purported pleural fluid analyte which at a cutoff value of 100µg/mL can diagnose MPM with a sensitivity of approximately 40% (37). In addition to developing novel MPM diagnostic tests, validation of this biomarker in a contemporary UK pleural population is therefore of substantial clinical value.

For validation of hyaluronan using McNemar’s, the sample size calculation assumes:

- 30% absolute increase in sensitivity
- Discordant proportion of 30% between MPM participants
- 80% power

- Two-sided alpha significance level of 5%

Under these assumptions, approximately 32 participants with confirmed MPM are required. Assuming a MPM prevalence of 7% (35) within the study population, this corresponds to a total recruitment target of approximately 457 participants.

The study will not recruit this number of participants within the one year timeframe proposed. We acknowledge that MPM analyses will therefore be exploratory. Estimates of diagnostic performance related to MPM may inform future studies.

A sample size of 150 participants as outlined in 7.1.1 may allow for analyses of pleural malignancy subtypes more prevalent than MPM, although these analyses will also be exploratory.

7.2 Data Analysis Strategy and Missingness

The primary outcome is defined as a final clinical diagnosis and biomarker analyses of the corresponding pleural effusion. The trial will be analysed primarily according to an intention-to-diagnose principle. It is not anticipated that many primary outcomes will be absent, as the trial is single patient contact and non-interventional reducing the likelihood of withdrawal from the study. However, if complete case missingness exceeds greater than 5%, per protocol analysis will also be completed.

Missingness is the absence of data needed to determine trial outcomes. This may be absent independent of the research, defined as missing at random (MAR), or possibly related to the research and how it is conducted, missing not at random (MNAR). Expected missingness for scientific analysis includes insufficient pleural fluid sample to run all analyses, loss of samples in transport or storage, or failure of scientific assays. These are expected to be MAR. For clinical outcomes, missingness is expected from death before diagnosis, loss of access to medical records (for example a participant moving out of the service area of UHMBT), or incomplete clinical documentation. Death before diagnosis may be MNAR, whereas the other items are likely to be MAR.

Patterns of missingness will be assessed using clinical review and statistical diagnostics; classification as MAR or MNAR will be agreed by the chief investigator and principal investigator.

Data that is considered MAR may be replaced with multiple imputation.

7.3 Diagnostic Performance Analyses

We anticipate that most research samples will come from the same pleural effusion sampling event as routine NHS cytology testing. We expect that effusion used for research will be homogenous with non-research pleural fluid used for NHS diagnostics. As such, any outputs from pleural fluid analysis where cytology is conducted on the same sample will be treated as paired for statistical purposes.

Some research samples will be collected from a different sampling event to that from which cytology results are generated, for example research samples collected via therapeutic thoracocentesis or interpleural drain insertion. If within 3 months of the closest cytology sampling event, we will count these as paired diagnostic comparisons as pleural pathology is unlikely to have changed significantly within this timeframe. We will record the proportion of participants for which the cytology event is greater than 3 months from research event. The time interval between biomarker sampling and cytology sampling will be recorded and explored as a covariate in regression models to assess whether sampling interval influences diagnostic concordance.

Pleural biopsy results will always be time discordant to pleural effusion sampling, and will be treated as accurately reflecting the same pleural pathology if within 3 months of the pleural effusion sampling.

New biomarkers are expected to be ELISA or flow cytometry based. Flow cytometry data is typically binary for each receptor that is detected, for example high and low, or present or absent. However, it is likely 2 or more receptors will be needed to give diagnostic accuracy, therefore creating 4, 9, 16 or higher numbers of categorical groups. ELISA is likely to yield continuous data on concentrations of the detected molecule(s).

Biomarkers may be combined to form a panel, potentially spanning both flow cytometry and ELISA methodologies. Biomarkers and biomarker combinations may be interpreted as binary (malignancy or not), or divided into risk groups, for example high risk, moderate risk, and low risk.

Clinical outcomes will be both binary, malignancy or not, and recorded individually e.g. para-pneumonic effusion, lung adenocarcinoma, MPM. Clinical outcomes will be determined by the clinical care team using all available clinical, radiological, and histological information independent of the research. Clinical outcomes will be recorded by researchers several months after the sample was collected to allow clinical outcome to become apparent. If a final diagnosis, or other clinical outcomes data, is not known at the first access of medical records, researchers will access these again at a later date until a final diagnosis is known or the study ends.

Biomarkers will be competing against cytology results primarily, and secondarily biopsy results (the current 'gold standard').

We anticipate cytology results to either diagnose a specific cancer, use non-descript terminology such as atypical mesothelial proliferation, or be reported as normal. For the purposes of assessing biomarker validity, cytology results will be handled as binary; either diagnosing a malignancy or not. For example, indeterminate cytology reports will be categorised as negative. This reflects clinical decision-making, where indeterminate cytology does not confirm malignancy. Pleural biopsy results will be treated similarly.

Although sensitivity is the primary accuracy parameter, potential biomarkers will also undergo specificity, positive predictive value and negative predictive value calculations, with contingency tables generated. Likelihood ratios will be calculated. A receiver operating characteristic (ROC) curve will be plotted and discrimination, the area under the ROC curve (AUC), will be calculated.

7.4 Non-Inferiority and Superiority

All novel and existing biomarkers will initially be evaluated using non-inferiority testing against cytology, with superiority testing performed where sample size and effect estimates permit.

Non-inferiority will be concluded if the lower bound of the two-sided 95% confidence interval for the difference in sensitivities (biomarker minus cytology) lies above -5%. Superiority will be concluded if the 95% confidence interval for the difference in sensitivities excludes 0 in favour of the biomarker.

For binary outcomes, McNemar's test will be used to estimate paired differences in sensitivity, with non-inferiority and superiority determined using confidence intervals rather than p-values. For continuous biomarker outputs, DeLong's test will be used to assess differences in discrimination (AUC).

7.5 Time to Event Analyses

Time-to-event analyses will be undertaken to assess whether biomarker availability may accelerate diagnostic pathways.

Time to diagnoses outcomes will include:

- Time from biomarker result hypothetical availability to final diagnosis
- Time from biomarker result hypothetical availability to cytology result availability
- Time from biomarker result hypothetical availability to pleural biopsy result availability

Survival analyses will be conducted using Kaplan-Meier curves for each malignant diagnosis, stratified by biomarker status (positive vs negative, and risk strata), with all-cause mortality as the primary survival endpoint.

To evaluate whether biomarkers independently shorten time to diagnosis, Cox proportional hazards regression will be performed, adjusting for relevant clinical covariates. Proportional hazards assumptions will be formally assessed.

7.6 Clinical Context and Model Performance

We recognise the importance of going beyond AUC, and biomarker performance will be evaluated within a broader clinical decision-making framework.

To achieve this, calibration will be assessed using:

- Calibration plots
- Calibration slope and intercept
- Brier score

These metrics will evaluate whether predicted risks align with observed outcomes and whether models over- or under-estimate malignancy risk. Multi-marker panels will be developed utilising penalised regression models, for example elastic net, reducing the likelihood of overfit and improving generalisability.

Models will be internally validated using bootstrapping to estimate optimism-adjusted performance. These models will incorporate:

- Biomarker analyses
- Pleural fluid biochemistry
- Microbiology
- Cytology

Odds ratios with 95% confidence intervals will be reported. Model discrimination will be assessed using AUC and DeLong's test to compare discrimination.

Decision curve analysis will be used to quantify the net clinical benefit of biomarkers across a range of clinically relevant threshold probabilities, comparing:

- Biomarker(s) alone
- Standard pleural fluid analysis
- Standard pleural fluid analysis plus pleural biopsy
- Combined diagnostic strategies

Exploratory analyses may include net reclassification improvement and integrated discrimination improvement, interpreted cautiously.

7.7 Targeted Use of Hyaluronan in Indeterminate Pleural Cytology

The previously reported high specificity of pleural fluid hyaluronan for MPM, reported to be as high as 99.3% at a cut-off of 150 µg/mL (37), suggests a potential role in risk stratification and diagnostic prioritisation in selected high-risk populations. In participants with a sufficient latency period following asbestos exposure, an exudative pleural effusion, and cytology reported as atypical mesothelial proliferation, we will explore whether hyaluronan concentrations have clinical utility.

7.8 Other data analysis

The collected clinical dataset will constitute a valuable resource for pleural effusion epidemiology, diagnostic pathways, and survival outcomes. We plan to approach these data with targeted questions and hypotheses. We may compare to or complement our findings with publicly available data such as NHS Fingertips.

Flow cytometry and single-cell RNA sequencing findings will be compared with publicly available reference datasets, including the Mesothelial Cell Atlas (http://mesothelialcellatlas.com/), to contextualise molecular findings within existing biological frameworks.

8 ETHICAL and REGULATORY CONSIDERATIONS

8.1 Assessment and management of risk

As the isolated samples are unscreened, there is a risk to research staff from potential infectious agents, these risks are mitigated by the use of controls, please see appended risk assessment.

8.2 Research Ethics Committee (REC) review

The study is sponsored by Lancaster University, who have undertaken a governance review, and will seek a favourable opinion from an NHS REC and HRA approval review prior to commencement. A research governance review has also been undertaken at UHMBT. All correspondence with the NHS REC will be retained.

An annual progress report (APR) will be submitted to the NHS REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended. It is the responsibility of the chief investigator and principal investigator to produce the APR.

The chief investigator or principal investigator will notify the NHS REC at the end of the study. If the study is ended prematurely, the chief investigator or principal investigator will notify the REC, including the reasons for the premature termination. Within one year after the end of the study, the chief investigator or principal investigator will submit a final study report with the results, including any publications/abstracts, to the REC.

8.3 Clinical Research

The study will follow the UK Policy Framework for Health and Social Care Research. This includes being in concordance with the Data Protection Act (2018) and General Data Protection Regulations regarding the handling of confidential patient information. Processing confidential clinical data on participants will be justified by first, achieving a favourable opinion from an NHS REC review, and second by obtaining their explicit consent, in accordance with English common law principles.

8.4 Human Tissue

Pleural samples are ‘tissues from the living’ specifically listed as ‘relevant material’ by the UK Human Tissue Authority (HTA). As the research is being carried out in England, there are obligations relating to the Human Tissue Act (2004). To comply with this legislation, samples used for research or clinical trials would normally need to be processed at a facility holding a HTA license.

UHMBT holds a HTA license at both Royal Lancaster Infirmary and Furness General Hospital for post-mortem examinations. It does not however hold a HTA license for research. Lancaster University does not possess a HTA license of any format.

For UHMBT, an exception applies as the samples are being held ‘incidental to transportation’. Regarding Lancaster University, it is intended that the study will achieve a favourable opinion from a recognised REC as previously discussed. NHS REC approval will exempt the need of a HTA license for the duration of the project. However, samples must be disposed of or transferred to an institution with a HTA license at the end of the project.

For non-fixed samples which are used for molecular biology techniques, such as genomics, material may be rendered acellular, for example by creating a cDNA library. Once rendered acellular samples would no longer fall within the restrictions of the HTA.

Relevant material used for research also needs the consent of the patient from which they are collected, which will be obtained explicitly before participation in this research.

We intend to culture immune and mesothelial cells and then analyse the culture medium to assess the cells and molecules secreted by these cells. This will allow attribution of secreted molecules to specific cell types. Cultured cells would be relevant material and would fall under the auspices of the HTA, with the exemptions listed above applying here also. Cells would be cultured in containment level 2 culturing facilities at Lancaster University and disposed according to standard operating procedures after analysis. We will not establish in vitro cell lines from these samples.

8.5 Regulatory Review & Compliance

Before any site can enrol patients into the study, the study management group or their designee will ensure that appropriate approvals from participating organisations are in place.

The protocol will be submitted to an appropriate independent expert for peer review.

8.6 Patient & Public Involvement

Patient and public involvement (PPI) has been undertaken prior to study commencement to ensure the acceptability of the consent process, use of clinical data, and the overall research design. We aim to have yearly interactions with a PPI focus group to relay preliminary outcomes and facilitate co-design of project direction.

8.8 Indemnity

Lancaster University will act as the study sponsor. Lancaster University's legal liability and research insurance will apply for harm arising from the management and design of the study. UHMBT's NHS indemnity will apply for harm arising from study conduct that occurs at UHMBT.

9 STUDY MANAGEMENT

9.1 Study Start Up

Protocol training will be given to UHMBT research staff via site induction visit (SIV) before research commences. Slides of the SIV will be provided to UHMBT research staff for future reference, along with contact details for the study management group.

9.2 Protocol compliance

The chief investigator and principal investigator will be responsible for ensuring protocol compliance. Specifically, the chief investigator will ensure compliance with the activities undertaken at Lancaster University, and the principal investigator will ensure compliance with the activities undertaken at UHMBT.

Accidental protocol deviations can happen at any time. They will be adequately documented and reported to the chief investigator or principal investigator immediately, and where necessary reported to the sponsor. Deviations from the protocol which are found to frequently recur are not acceptable and will require immediate action from the chief investigator or principal investigator.

9.3 Interim Monitoring Visits

The chief investigator or co-investigator will meet with UHMBT research delivery team and principal investigator or co-principal investigator once every 12 months to discuss research progression and any queries from the site. Compliance with the protocol, case report form and safety reporting will also be checked. The Sponsor may also conduct triggered or scheduled monitoring of the study document, and will request study progression updates on a regular basis.

9.4 Data Management Plan

A full position on data management is given in the appended data management plan.

9.5 Access to the final study dataset

The study management group and any individual they delegate to will have access to the final study dataset. The pseudonymised final study data set, or parts thereof, will be uploaded to publicly available data repositories. Reasonable requests to access these data will be granted at the discretion of the study management group. Published articles may link to these public repositories.

9.6 Dissemination policy

The data arising from the study will be owned by Lancaster University. On completion of the research a Final Study Report will be prepared. Any participants that wish to may request a copy.

The study management group will have the rights to determine the content and dissemination of any data related to the study. It is envisaged that findings of the study will be published in a peer-reviewed journal and presented at medical or scientific conferences. The study's findings may be split into results regarding biomarker validation and pleural effusion epidemiology aimed at medical journals and conferences, and basic science insights into pathophysiology of pleural effusion aimed at scientific journals or conferences.

9.6.1 Authorship eligibility guidelines

The chief investigator and principal investigator will determine the position of authors on any disseminated work. Researchers can qualify for authorship if they meet the criteria of the journals that findings may be reported in, but generally it is anticipated that one or more of the following non-exhaustible list would lead to authorship:

- Recruiting participants and performing pleural procedures which lead to sample collection.
- Making significant contributions to collection of corresponding clinical data.
- Completing bioscience techniques on collected samples that generate publishable data.
- Making significant contributions to data analysis.
- Drafting and reviewing significant portions of any published manuscripts.

10 REFERENCES

1. Ferreiro L, Toubes ME, Suárez-Antelo J, Rodríguez-Núñez N, Valdés L, Ferreiro L, et al. Clinical overview of the physiology and pathophysiology of pleural fluid movement: a narrative review. ERJ Open Research. 2024–09–30;10(5).
2. Karpathiou G, Stefanou D, Froudarakis ME. Pleural neoplastic pathology. Respiratory Medicine. 2015/08/01;109(8).
3. Karpathiou G, Péoc'h M, Sundaralingam A, Rahman N, Froudarakis ME, Karpathiou G, et al. Inflammation of the Pleural Cavity: A Review on Pathogenesis, Diagnosis and Implications in Tumor Pathophysiology. Cancers 2022, Vol 14, Page 1415. 2022–03–10;14(6).
4. Light RW. Pleural Effusion. New England Journal of Medicine. 2002–06–20;346(25).
5. Yu Y-X, Yang Y, Wu Y-B, Wang X-J, Xu L-L, Wang Z, et al. An update of the long-term outcome of patients with nonspecific pleurisy at medical thoracoscopy. BMC Pulmonary Medicine 2021 21:1. 2021–07–12;21(1).
6. Lucà S, Pignata G, Cioce A, Salzillo C, Cecio RD, Ferrara G, et al. Diagnostic Challenges in the Pathological Approach to Pleural Mesothelioma. Cancers 2025, Vol 17, Page 481. 2025–02–01;17(3).
7. Clive AO, Kahan BC, Hooper CE, Bhatnagar R, Morley AJ, Zahan-Evans N, et al. Predicting survival in malignant pleural effusion: development and validation of the LENT prognostic score. Thorax. 2014–12–01;69(12).
8. Sriram KB, Relan V, Clarke BE, Duhig EE, Yang IA, Bowman RV, et al. Diagnostic molecular biomarkers for malignant pleural effusions. Future Oncology. 2011/06/01;7(6).
9. Churg A. New developments in mesothelial pathology. Histopathology. 2024/01/01;84(1).
10. Dacic S. Pleural mesothelioma classification—update and challenges. Modern Pathology. 2022/01/01;35(S1).
11. Karpathiou G, Hathroubi S, Patoir A, Tiffet O, Casteillo F, Brun C, et al. Non-specific pleuritis: pathological patterns in benign pleuritis. Pathology. 2019/06/01;51(4).
12. Arnold DT, Fonseka DD, Perry S, Morley A, Harvey John E, Medford A, et al. Investigating unilateral pleural effusions: the role of cytology. European Respiratory Journal. 2018–11–08;52(5).
13. Kassirian S, Hinton SN, Cuninghame S, Chaudhary R, Iansavitchene A, Amjadi K, et al. Diagnostic sensitivity of pleural fluid cytology in malignant pleural effusions: systematic review and meta-analysis. Thorax. 2023–01–01;78(1).
14. Hsu S-C, Chang S-Y, Hwang Y-T, Terng H-J, Tsai C-L, Shen C-H, et al. mRNA markers associated with malignant pleural effusion. Scientific Reports 2023 13:1. 2023–04–24;13(1).
15. Liang C, Liu N, Zhang Q, Deng M, Ma J, Lu J, et al. A detection panel of novel methylated DNA markers for malignant pleural effusion. Frontiers in Oncology. 2022/09/13;12.
16. Yang Z, Song Z, Chen Z, Guo Z, Jin H, Ding C, et al. Metabolic and lipidomic characterization of malignant pleural effusion in human lung cancer. Journal of Pharmaceutical and Biomedical Analysis. 2020/02/20;180.
17. Palstrøm NB, Overgaard M, Licht P, Beck HC, Palstrøm NB, Overgaard M, et al. Identification of Highly Sensitive Pleural Effusion Protein Biomarkers for Malignant Pleural Mesothelioma by Affinity-Based Quantitative Proteomics. Cancers 2023, Vol 15, Page 641. 2023–01–19;15(3).
18. Porcel JM, Porcel JM. Biomarkers in the diagnosis of pleural diseases: a 2018 update. Therapeutic Advances in Respiratory Disease. 2018–10;12.
19. Zhang M, Yan L, Lippi G, Hu Z-D. Pleural biomarkers in diagnostics of malignant pleural effusion: a narrative review. Translational Lung Cancer Research. 2021 Mar;10(3).
20. Javadi J, Dobra K, Hjerpe A, Javadi J, Dobra K, Hjerpe A. Multiplex Soluble Biomarker Analysis from Pleural Effusion. Biomolecules 2020, Vol 10, Page 1113. 2020–07–28;10(8).
21. Love K, Costa RMd, Beckmann M, Morley A, Bhatnagar R, Maskell N, et al. Metabolomics differentiate cancer from non-cancer pleural effusions based on steroid lipids and acyl carnitines. medRxiv. 2020–07–29.
22. Churg A, Galateau-Salle F, Roden AC, Attanoos R, Thusen JHvd, Tsao M-S, et al. Malignant mesothelioma in situ: morphologic features and clinical outcome. Modern Pathology. 2020/02/01;33(2).
23. Cortes-Dericks L, Schmid RA. CD44 and its ligand hyaluronan as potential biomarkers in malignant pleural mesothelioma: evidence and perspectives. Respiratory Research. 2017 Apr 12;18(1).

24. Tanaka K, Osada H, Murakami-Tonami Y, Horio Y, Hida T, Sekido Y. Statin suppresses Hippo pathway-inactivated malignant mesothelioma cells and blocks the YAP/CD44 growth stimulatory axis. Cancer Letters. 2017/01/28;385.
25. Vidergar R, Balduit A, Zacchi P, Agostinis C, Mangogna A, Belmonte B, et al. C1q–HA Matrix Regulates the Local Synthesis of Hyaluronan in Malignant Pleural Mesothelioma by Modulating HAS3 Expression. Cancers 2021, Vol 13, Page 416. 2021–01–22;13(3).
26. Balduit A, Vidergar R, Zacchi P, Mangogna A, Agostinis C, Grandolfo M, et al. Complement protein C1q stimulates hyaluronic acid degradation via gC1qR/HABP1/p32 in malignant pleural mesothelioma. Front Immunol. 2023 Jun 2;14.
27. AK E, AH R, LE R, EV N, BM M, KL S. LYVE-1-expressing Macrophages Modulate the Hyaluronan-containing Extracellular Matrix in the Mammary Stroma and Contribute to Mammary Tumor Growth - PubMed. Cancer research communications. 05/31/2024;4(5).
28. Zhang N, Kim SH, Gainullina A, Erlich EC, Onufer EJ, Kim J, et al. LYVE1+ macrophages of murine peritoneal mesothelium promote omentum-independent ovarian tumor growth. Journal of Experimental Medicine. 2021/12/06;218(12).
29. Sheng R, Yin Y, Wang X. Mesothelial and immune cells interplay in the tumor microenvironment. Trends in Molecular Medicine. 2025;0(0).
30. Jazwinska DE, Kulawiec DG, Zervantonakis IK. Cancer-mesothelial and cancer-macrophage interactions in the ovarian cancer microenvironment. American Journal of Physiology-Cell Physiology. 2023 Aug 31;325(3).
31. Panou V, Bhatnagar R, Rahman N, Christensen TD, Pietersen PI, Arshad A, et al. Advances in the diagnosis and follow-up of pleural lesions: a scoping review. Expert Review of Respiratory Medicine. 2024–06–02;18(6).
32. Huang Z-Y, Shao M-M, Zhang J-C, Yi F-S, Du J, Zhou Q, et al. Single-cell analysis of diverse immune phenotypes in malignant pleural effusion. Nature Communications 2021 12:1. 2021–11–18;12(1).
33. Jeong H-o, Lee H, Kim H, Jang J, Kim S, Hwang T, et al. Cellular plasticity and immune microenvironment of malignant pleural effusion are associated with EGFR-TKI resistance in non–small-cell lung carcinoma. iScience. 2022/11/18;25(11).
34. Obacz J, Valer JA, Nibhani R, Adams TS, Schupp JC, Veale N, et al. Single-cell transcriptomic analysis of human pleura reveals stromal heterogeneity and informs in vitro models of mesothelioma. European Respiratory Journal. 2024–01–25;63(1).
35. Addala DN, Mercer R, Sundaralingam A, Iqbal B, Elsheikh A, Bedawi EO, et al. Incidence of Discordant Pleural Fluid Exudates and Diagnostic Patterns: A Retrospective Cohort Study. CHEST. 2025;0(0).
36. Darnton L. Mesothelioma by geographical area in Great Britain. 2025 July 2025.
37. Fujimoto N, Gemba K, Asano M, Fuchimoto Y, Wada S, Ono K, et al. Hyaluronic acid in the pleural fluid of patients with malignant pleural mesothelioma. Respiratory Investigation. 2013/06/01;51(2).