BMJ Open

# Diagnostic and prognostic biomarkers in the rational assessment of mesothelioma (DIAPHRAGM) study: protocol of a prospective, multi-centre, observational study

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2016-013324 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 04-Jul-2016 |
| Complete List of Authors: | Tsim, Selina; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Cancer Sciences<br>Kelly, Caroline; Cancer Research UK Glasgow Clinical Trials Unit<br>Alexander, Laura; Cancer Research UK Glasgow Clinical Trials Unit<br>McCormick, Carol; Cancer Research UK Glasgow Clinical Trials Unit<br>Thomson, Fiona; Cancer Research UK Glasgow Clinical Trials Unit<br>Woodward, Rosemary; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility<br>Foster, John; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility<br>Stobo, David; Queen Elizabeth University Hospital, Radiology<br>Paul, Jim; Cancer Research UK Glasgow Clinical Trials Unit<br>Maskell, Nick; University of Bristol, Academic Respiratory Unit, School of Clinical Sciences; North Bristol NHS Trust, Respiratory Research Unit<br>Chalmers, Anthony; University of Glasgow, Institute of Cancer Sciences; Beatson West of Scotland Cancer Centre<br>Blyth, Kevin; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Infection, Immunity and Inflammation |
| Primary Subject Heading: | Respiratory medicine |
| Secondary Subject Heading: | Oncology, Patient-centred medicine, Research methods |
| Keywords: | Mesothelioma, Biomarker, Diagnosis, Prognosis |
| | |

**TITLE:**

# DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN THE RATIONAL ASSESSMENT OF MESOTHELIOMA (DIAPHRAGM) STUDY: PROTOCOL OF A PROSPECTIVE, MULTI-CENTRE, OBSERVATIONAL STUDY

**AUTHORS:**

Selina Tsim[1/2], Caroline Kelly[3], Laura Alexander[3], Carol McCormick[3], Fiona Thomson[3], Rosie Woodward[4], John E Foster[4], David B Stobo[5], Jim Paul[3], Nick A Maskell[6], Anthony Chalmers[2/7], Kevin G Blyth[1/8]

**Affiliations**

1. Department of Respiratory Medicine, Queen Elizabeth University Hospital, Glasgow, U.K.
2. Institute of Cancer Sciences, University of Glasgow, U.K.
3. Cancer Research UK Glasgow Clinical Trials Unit, UK
4. Glasgow Clinical Research Imaging Facility, Queen Elizabeth University Hospital, Glasgow, U.K.
5. Department of Radiology, Queen Elizabeth University Hospital, Glasgow, U.K.
6. Academic Respiratory Unit, School of Clinical Sciences, University of Bristol, UK
7. Beatson West of Scotland Cancer Centre, Glasgow, UK
8. Institute of Infection, Immunology and Inflammation, University of Glasgow, U.K.

**Corresponding Author and Address**

Kevin G. Blyth

Consultant Respiratory Physician and NRS Career Research Fellow

Department of Respiratory Medicine, Administration Building,

Queen Elizabeth University Hospital,

Glasgow, G51 4TF

kevin.blyth@ggc.scot.nhs.uk

Tel: +44 141 451 6099

**Keywords:**

Mesothelioma

Biomarker

Diagnosis

Prognosis

**WORD COUNT:**

3998/4000

## ABSTRACT (300/300)

### INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer, which is difficult to diagnose. Thoracoscopy is frequently required but is not widely available. An accurate, non-invasive diagnostic biomarker would allow early specialist referral, limit diagnostic delays and maximize clinical trial access. Current markers offer insufficient sensitivity and are not routinely used. The SOMAmer® proteomic classifier and Fibulin-3 have recently demonstrated sensitivity and specificity exceeding 90% in retrospective studies. DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma) is a suitably powered, multi-centre, prospective observational study designed to determine whether these markers provide clinically useful diagnostic and prognostic information.

### METHODS AND ANALYSIS

Serum and plasma (for SOMAscan® and Fibulin-3, respectively) will be collected at presentation, prior to pleural biopsy/pleurodesis, from 83-120 MPM patients, 634-724 patients with non-MPM pleural disease and 109 asbestos-exposed controls. Final numbers of MPM/non-MPM cases will depend on the incidence of MPM in the study population (estimated at 13-20%). Identical sampling and storage protocols will be used in 22 recruiting centres and histological confirmation sought in all cases. Markers will be measured using the SOMAscan proteomic assay (SomaLogic Inc.) and a commercially available Fibulin-3 ELISA (USCN Life Science Inc.). The standard error in the estimated sensitivity and specificity will be <5% for each

marker and their performance will be compared to serum Mesothelin. Blood levels will be compared to paired pleural fluid levels and MPM tumour volume (using Magnetic Resonance Imaging) in a nested sub-study. The prognostic value of each marker will be assessed and a large bioresource created.

## ETHICS AND DISSEMINATION

The study has been approved by the West of Scotland Research Ethics Committee (Ref: 13/WS/0240). A Trial Management Group meets on a monthly basis. Results will be published in peer-reviewed journals, presented at international meetings and disseminated to patient groups.

**TRIAL REGISTRATION NUMBER:** ISRCTN10079972

## STRENGTHS OF THIS STUDY

- Prospective recruitment of patients at presentation with suspected pleural malignancy, reflecting when blood biomarkers would be drawn in clinical practice and avoiding potential confounding factors such as pleurodesis
- Strict sampling, processing and storage methods used in all patients
- Potential confounders including renal function, body weight and concomitant medications recorded
- All participants subject to rigorous diagnostics with a minimum of 12 months' follow-up in patients who do not have a histological or cytological diagnosis of malignancy
- A large bio-resource of serum, plasma, whole blood and pleural fluid, with prospectively collected detailed clinical information will be created

## LIMITATIONS OF THIS STUDY

- While the study design accounts for inherent diagnostic difficulties in Malignant Pleural Mesothelioma (MPM), with follow-up of patients with 'benign' pleural disease for a minimum of 12 months, there is likely to be a population of patients included in the study with suspected MPM who do not have a firm histological diagnosis. Every effort will be made in these cases to ascertain the post-mortem findings where available.
- Study dropout – there is likely to be a population of patients with MPM included in the study who will not be fit to return for follow-up clinic visits (or a study research visit) due to the natural history of the disease. In these patients it will not be possible to perform 3 month follow-up blood biomarker sampling (exploratory outcome). There is also likely to be a cohort of patients, e.g. who do not have a diagnosis of malignancy, who will become lost to follow-up.
- The final number of study participants who are diagnosed with MPM will not be known until the study completes recruitment. This results in a degree of uncertainty regarding the power available to test the primary hypotheses. The statistical analysis plan included in this manuscript takes into account this uncertainty. The protocol does however include an estimated final number of MPM based on audit data from several of the recruiting centres and number of MPM cases recruited during the course of the study will be kept under review by the Trial Management Group.

## INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an invasive thoracic malignancy, strongly associated with prior asbestos exposure. The median survival for patients with MPM is poor at 9-10 months [1,2]. However, the prognosis of individuals is highly variable and largely determined by histological subtype [2]. MPM frequently presents as an emergency with a large, symptomatic pleural effusion [3]. Early specialist referral is frequently required because pleural fluid aspiration cytology is unreliable [4] and histological confirmation is recommended in all patients [5]. Thoracoscopy (under local or general anaesthesia) [5], enables widespread tissue sampling [7] with diagnostic yields for malignancy >90% [6] but is not available in all centres. Thoracoscopy also allows pleurodesis or indwelling pleural catheter placement.

A reliable, non-invasive diagnostic biomarker for MPM would be a major clinical advance. This would allow clinicians to reliably differentiate likely MPM from secondary pleural malignancies (e.g. lung or breast cancer), which may present with similar clinical and imaging features but require less evolved diagnostic pathways. This reflects the improved sensitivity of pleural cytology in these diseases [8-10] and the frequent option of alternative sites for tissue biopsy. A positive MPM biomarker test could facilitate early referral to a thoracoscopy centre and avoid unnecessary diagnostic delay (e.g. due to repeated pleural aspirations), minimising the risk of subsequent needle-tract metastases [11,12] and maximizing opportunity for clinical trial enrolment. Previous studies have demonstrated that blood levels of single proteins,

including mesothelin [13,14], megakaryocyte potentiating factor (MPF) [15,16] and osteopontin [17], are higher in patients with MPM than in asbestos-exposed controls (AECs) and patients with secondary pleural malignancies. Mesothelin, a cell-adhesion glycoprotein that is over-expressed in MPM [18,19] is the most widely studied and is associated with an MPM sensitivity of 56-77% at 95% specificity [14,16,20]. However, a recent meta-analysis (of 4491 individuals (1026 with MPM)) reported a sensitivity of only 32% at 95% specificity. Mesothelin does not, therefore, contribute to current diagnostic algorithms [21]. MPF offers no advantage over mesothelin [16], while the clinical utility of osteopontin is limited by stability and reproducibility concerns [17].

An ideal MPM biomarker would be measurable in blood for ease of collection and offer sufficient sensitivity at high specificity in patients presenting with suspected MPM. Differentiation between advanced disease patients and appropriate controls is of limited value. High specificity is mandatory for a low prevalence disease, and should apply to patients with asbestos exposure and non-MPM pleural disease. Biomarker results should also correlate with disease extent and have defined relationships with potential confounders including renal function [22] and the effect of pleural interventions. The latter is important because the precedent has been established in prostate [23,24] and breast cancer [25], that recent sampling, resection or peri-tumoural inflammation may affect biomarker expression. This is particularly relevant to MPM where biopsies are frequently large and often combined with pleurodesis. Several previous biomarker studies, which validated

inconsistently in external populations, used samples acquired at later time-points, often post-diagnosis (and post-pleurodesis) including samples taken prior to, during, or after resection surgery [17,26,27]. The aim of the DIAPHRAGM study is to prospectively evaluate the diagnostic and prognostic performance of the SOMAscan proteomic classifier [28] and fibulin-3 [26], which have demonstrated high sensitivity and specificity in recent retrospective series. The study has been designed to generate clinically meaningful results, which can be related to MPM biology and confounding factors, and applied to patients at first presentation.

**SOMAmer-based Proteomic Classifier**

The SOMAscan assay is a highly multiplexed proteomic platform that utilizes SOMAmer (Slow Off-rate Modified Aptamers) reagents to selectively bind and quantify proteins [29]. A 13-protein classifier was developed by SomaLogic Inc. (Boulder, Colorado), using this novel proteomics-based biomarker detection technique [28] in a retrospective study over 800 proteins were measured in the serum of 117 MPM patients and 142 AECs, collected at surgical MPM centres in the US between 1996 and 2011. Using a panel of 13 differentially expressed proteins and a cut-point of 0.5, the classifier was able to segregate MPM from controls with an area under the curve (AUC) of 0.99 +/- 0.01 in training (60 MPM/60 controls), 0.98 +/- 0.04 in blinded verification (19 MPM/20 controls) and 0.95 +/- 0.04 in blinded validation sets (38 cases/62 controls) [28]. The combined sensitivity for the three cohorts was 93% at 91% specificity. Based on the published ROC curve for the validation cohort, sensitivity at 95% specificity appeared to be approximately 78%,

although the authors did not report this value. This performance exceeds that of any previous MPM biomarker, although the classifier's specificity appeared lower in patients with non-MPM pleural effusion (n=32). There was a modest correlation between classifier score and disease stage, but prognostic significance was not assessed. The 13 classifier proteins (nine up-regulated, four down-regulated) have not previously been associated with MPM. Their functions fall into two broad groups; regulation of proliferation and inflammation. Quite apart from their biological relevance to MPM, the latter is an important potential confounder because many of the patients involved will have previously undergone pleurodesis. In addition, several groups have reported an independent interaction between prognosis and inflammatory biomarkers in MPM, including neutrophil-to-lymphocyte ratio (NLR) [30-32], monocytosis [33] and the modified Glasgow Prognostic Score [32]. Therefore, adequate understanding of the diagnostic and prognostic utility of this assay requires replication in a pre-pleurodesis cohort and prospective evaluation of interactions between inflammatory biomarkers and SOMAscan scores.

**Fibulin-3**

Fibulin-3 is a secreted glycoprotein, encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [34]. Fibulin-3 is over-expressed in MPM tumours relative to adjacent benign pleura [26] and expressed and secreted by MPM cell lines [27]. Pass et al retrospectively measured fibulin-3 in the plasma of 92 MPM patients, 136 AECs, 93 patients with non-MPM pleural effusion and 43 healthy controls [26].

A plasma cut-point of 52 ng/ml provided 97% sensitivity at 95% specificity and a 95% CI of the AUC of 0.97-0.99 in differentiating MPM from all other cases. However, in a blinded external validation set, sensitivity was below 40% (at 95% specificity), with an AUC=0.87.

Subsequent studies have revealed mixed results. In a study of 153 patients (82 with MPM), Creaney et al reported a sensitivity of 22% (at 95% specificity) at the same 52 ng/ml cut-point and an AUC of 0.671 (0.606 to 0.732), which was significantly inferior to mesothelin measured in the same patients (sensitivity 56% (at 95% specificity); AUC 0.816 (0.755 to 0.867)) at a 2.5 nM threshold [14]). In a small Egyptian study using an unspecified Fibulin-3 assay and internally-defined cut-points, Agha et al reported 100% sensitivity/78% specificity in differentiating MPM cases (n=25) from non-malignant pleural disease (n=9), and 88% sensitivity/82% specificity in differentiating MPM from secondary pleural malignancies (n=11) [35]. No combined sensitivity was reported. An Italian study found no difference in Fibulin-3 levels but used serum (not plasma), a control group without pleural disease (Asbestosis) and contained only 14 patients with MPM [36].

## METHODS AND ANALYSIS

### Study Design

DIAPHRAGM is a prospective, multi-centre observational study. The study incorporates sampling windows that correspond to the proposed use of a diagnostic biomarker, i.e. at presentation with Suspected Pleural Malignancy (SPM). The overall study design is summarized in Figure 1. The main impact

of this design is that biomarkers will be drawn before a diagnosis is made. In addition to better replicating the future use of these markers, this avoids the potential confounding effect of pleurodesis on biomarker results. The diagnostic performance of the SOMAmer panel and Fibulin-3 will be assessed using cut-points determined in the relevant original studies and compared to mesothelin. Identical processing and storage protocols will be used in patients with SPM and a group of AECs (see Figure 2). Potential confounders including renal function, inflammatory indices and drugs will be recorded at all visits. An exploratory, cross-sectional Magnetic Resonance Imaging (MRI) sub-study will determine if there is any correlation between blood biomarker levels and MPM tumour volume, as has been established for Mesothelin using Computed Tomography-Positron Emission Tomography scanning [37].

**Study Objectives and Outcome Measures**

These are presented in Table 1.

**Setting**

At least 737 consecutive patients with SPM will be recruited from 21 centres (20 in the UK, 1 in Republic of Ireland). These are a mixture of academic and more clinically orientated units. This should make the results of the DIAPHRAGM study generalizable to patients presenting with SPM to acute hospital services. The principal criterion used to select centres was that they had sufficiently evolved pleural diagnostic services to deliver a reliable diagnosis. Specifically, access to on-site thoracoscopy (ideally including local

anaesthetic thoracoscopy (LAT)) and a regional mesothelioma MDT meeting (for diagnostic review and staging) was required.

**Screening and Eligibility Assessment**

Suspected Pleural Malignancy

Cases will be identified on presentation to a Respiratory out-patient clinic or acute hospital admissions unit. This will be based on the history, examination and available investigations. Potentially eligible patients will be provided with the study Patient Information Sheet (PIS, see Online Supplementary Appendix 1) and eligibility assessed based on the following criteria:

*Inclusion Criteria:*

- SPM, defined by a unilateral pleural effusion or pleural mass lesion
- Sufficient fitness for diagnostic sampling (site investigator's clinical judgment)
- Informed written consent

*Exclusion Criteria:*

- Intercostal chest drain in-situ, or inserted within the previous 3 months

Asbestos-related pleural plaques are not an inclusion criterion since these are absent in up to 25% of MPM cases [38], and are also common in asbestos-exposed populations without MPM [39]. Patients with lung nodules or other visceral mass lesions are not excluded, assuming the investigator suspects pleural malignancy. This is because of the high prevalence of lung nodules in

the target population (older patients, commonly smokers) and the high false positive rate of CT imaging in this regard [40].

Subjects recruited to the SPM arm will generate cohorts of MPM and non-MPM pleural disease of various aetiologies, likely including Benign Asbestos-related Pleural Effusion and secondary pleural malignancies. These numbers will be sufficient to address the primary objective with sufficient statistical power to inform clinical practice (see later section).

Asbestos-exposed control (AEC) subjects

109 AECs will be recruited via invitations sent by Clydeside Action on Asbestos (CAA), an advocacy body based in Glasgow with a database of over 600 clients, or by Respiratory clinics at the host centre. Individuals will be invited to participate by letter (if identified via CAA) or given the PIS (see Online Supplementary Appendix 2) at clinic. All subjects will be invited to a single research clinic visit assuming the following Eligibility Criteria are met.

*Inclusion Criteria:*

- Documented history of asbestos exposure and associated pleural plaques, asbestosis or diffuse pleural thickening
- Willing and able to travel to a research clinic interview in Glasgow
- Informed written consent

*Exclusion Criteria:*

- Known MPM
- Known or suspected other thoracic malignancy under investigation

- Known pleural effusion of any cause

Cross-sectional MRI sub-study

50 patients will be recruited to address the study's exploratory objectives (see Table 1). Eligibility will be determined based on the following criteria.

*Inclusion Criteria:*

- Pleural histological sampling (by LAT/image-guided biopsy) indicated to investigate SPM following a non-diagnostic pleural aspiration
- Recruited in a West of Scotland centre

*Exclusion Criteria:*

- Unable to undergo MRI (claustrophobia or known contraindications such as pacemaker, ferrous metal implants or foreign body)
- Allergy to Gadolinium contrast
- Renal impairment (eGFR <30ml/min)
- Pregnancy

Based on previous audit data from the host centre we expect at least 40% (n=20) of patients in the sub-study to have MPM. Eligible subjects will be approached at the clinical visit during which non-diagnostic pleural aspiration results, and the need for further investigation, are discussed. Subjects will be provided with a separate PIS (see Online Supplementary Appendix 3) and will be asked to provide additional informed written consent.

## Consent

All subjects will be given sufficient time (as judged by themselves) to provide written informed consent after reading the relevant PIS and having the opportunity to ask questions.

## Outcome Measures

The outcome measures associated with each of the trial's objectives are detailed in Table 1.

*Final Diagnosis*

A specific cytological or histological pleural diagnosis will be sought in all patients according to national guidelines [21]. This will be recorded as the Final Diagnosis, which may be based on immediate repeat biopsies felt to be indicated by the site PI (see Figure 1). Any cytologically or histologically confirmed non-MPM diagnosis (e.g. pleural metastases from lung cancer) will be recorded without the need for any further updates. However, sites will need to provide updates for any non-MPM diagnosis that is not cytologically or histologically confirmed (e.g. parapneumonic effusion). These will be submitted on the 12-month anniversary of the original diagnosis, or as soon as any new pleural diagnosis is made. This aims to capture any false negative diagnostic tests from the initial presentation, acknowledging the major diagnostic challenges posed by pleural malignancies, particularly MPM.

*Biomarker Sampling and Storage*

Blood samples (+/- pleural fluid in WoS centres) will be drawn and immediate processing performed at each study centre. Samples can be taken before or after pleural aspiration. Patients with positive pleural cytology cannot be recruited (see Figure 1(a)). Duplicate samples will be collected for all measurements at all visits, ensuring redundancy in case of loss or damage to samples during transportation to the appropriate central laboratory. SOMAmer biomarker levels will be measured in serum; therefore, 9 ml of venous blood will be collected first into a vacutainer tube containing SST clot activator. Fibulin-3 levels will be measured in plasma; therefore, 9 ml of venous blood will be collected second into a vacutainer tube containing EDTA. In centres contributing to the exploratory MRI sub-study (WoS sties only) 20 ml of pleural fluid will be also collected into a plain container if pleural fluid is being drawn for diagnostic/therapeutic purposes at the same visit.

*Biomarker Processing and Storage*

Serum samples will be allowed to clot for 30 minutes before centrifugation. Plasma and pleural fluid samples will be centrifuged immediately. All samples will be centrifuged at 2200g for 15 minutes at room temperature. For all samples, the supernatant will be withdrawn by pipette, aliquoted into cryovials of at least 250µL volume, labeled and placed into a -80 freezer within 2 hours. Samples will be stored at each recruiting centre until batched transport to the appropriate study laboratory.

*Biomarker Analyses*

SomaLogic Inc. (Boulder, Colorado, USA) will perform all SOMAscan proteomic analyses [28]. This utlises SOMAmer reagents to specifically bind to protein targets in blood. Relative protein concentrations will be converted to measurable nucleic acid signals that are quantified by hybridization to DNA microarrays [29].

Fibulin-3 and mesothelin levels will be measured using ELISA methods validated according to the FDA-recommended guidelines for bioanalytical methods [41]. Fibulin-3 levels will be measured using the commercially available ELISA (USCN Life Science Inc, Wuhan, China) as in the original Pass study [26]. Mesothelin will be measured using the Mesomark ELISA (Fujirebio Diagnostics, Inc, PA, USA). In parallel, we aim to develop a custom multiplex ELISA assay that has the potential to simultaneously measure multiple biomarkers (fibulin-3, mesothelin and osteopontin) with greater accuracy (U-PLEX, Meso Scale Diagnostics, Rockville, USA)

*Magnetic Resonance Imaging*

Patients will be scanned at the Queen Elizabeth University Hospital, Glasgow, on a 3.0T Siemens Verio MRI Scanner. After localisation of the affected thoracic cavity, an isotropic 3D T1-weighted volume will be acquired using VIBE sequences. A stack of axial slices covering the entire lung and surrounding pleura will be acquired as a set of short breath-holds. Gd-DTPA contrast (Gadovist) will be administered via a peripheral intravenous line as a 15-40 ml bolus (0.05 mmol/kg). VIBE sequences will be reacquired at copied

slice positions to provide pre and post-contrast images. The total scan time will be around 45 minutes. Regions of enhancing pleural tumour will be defined using semi-automated signal intensity thresholding based on contrast-enhanced axial slices using Myrian Intrasense™ software.

*Survival*

Survival will be recorded in days from the date of study registration to the data of death, from any cause.

**Sample Size, Assumptions and Uncertainties**

Sample size estimations for each marker were based on published data at the point of study design and a projected MPM incidence of 13-20% in the SPM cohort. The power available to test the hypotheses below is therefore reported as a range, based on final MPM numbers lying between 83 (13% incidence) and 120 (20% incidence).

Primary Objective

*SOMAscan Assay*

We hypothesize that the MPM sensitivity and specificity exceed 90%, based on previously reported performance in combined training, verification and validation sets (sensitivity 93.2% (88.6–97.7%), specificity (90.8% (86.1–95.6%) [28]). Recruitment of 83-120 MPM patients will allow us to distinguish a sensitivity of >90% from a sensitivity <80% with 80-93% power, respectively, at the 5% 1-sided level of significance. 83-120 MPM patients will allow discrimination between a specificity <80% and a specificity >90%, with

80-88% power at the 5% 1-sided level of statistical significance. The standard error in the estimated sensitivity and specificity will be less than 5%, across all possible outcomes.

*Fibulin-3*

We hypothesize that the MPM sensitivity will exceed 80% and that the specificity will exceed 90% (at the 52 ng/ml cutoff). These figures are based on a reduced level of performance to the primary results reported by Pass et al (97% sensitivity, 95% specificity), given lower sensitivity in the external validation cohort studied (40% at 95% specificity) [26].

With 83-120 MPM patients the study will be able to distinguish a sensitivity of >80% from a sensitivity <70% with 65-80% power, respectively, at the 5% 1-sided level of statistical significance. The standard error in the estimated sensitivity will be less than 5%. In order to achieve 90% power to distinguish a specificity of >90% from a specificity <85% at the 5% 1-sided level of statistical significance, a random sample of 378 non-MPM samples will be analysed. The standard error in the estimated specificity will be <2.3%.

The study data will be used to estimate the AUC for the SOMAscan marker for distinguishing MPM from non-MPM patients in the SPM cohort. Assuming 83-120 patients in the MPM group and 83-120 in the non-MPM group the AUC can be estimated with a 95% confidence interval of width 0.120-0.168 (assuming a cut-point exists with a reasonable sensitivity of 80% and a modest specificity of 40%). If more sensitive/specific cut-points exist the width

of the 95% confidence interval will be much reduced. The study data will be used to develop a new diagnostic signature based on Fibulin-3 and SOMAscan results to distinguish MPM from non-MPM effusions.

Secondary Objectives

The study data will be used to determine whether baseline SOMAscan results and/or fibulin-3 levels, or a change in levels at 3 months (Fibulin-3 only), are independent prognostic factors for MPM. A correlation of 0.4 between existing prognostic factors and each marker has been assumed. For the baseline levels, to detect an approximate doubling in median OS (from 6 month to 12 months - a hazard ratio of 2) with 80% power and 5% 2-sided level of statistical significance between a good/poor prognostic group based on dichotomising these markers requires at least 83 MPM patients recruited over three years with approximately 6 months subsequent follow-up to observe 66 deaths. For the 3-month change levels, a hazard ratio of 2.38 can be detected (80% power, 5% 2-sided level of statistical significance) when 49 deaths are observed in the estimated 66 out of 83 patients who survive to 3 months.

Exploratory Objectives

These will be addressed in the MRI sub-study, which will generate a sample of at least 20 MPM patients. This will allow moderately large associations (0.6) between the exploratory outcome measures (see Table 1) to be detected at 80% power at the 5%, two-sided level of statistical significance. The effect of pleural biopsies +/- drainage/pleurodesis on Fibulin-3 levels will be

assessed using all 50 patients recruited. This will allow moderately small differences (standardised difference of 0.4) to be detected with 80% power at the 5% two-sided level of statistical significance.

**Statistical Analysis Plan**

Primary Analysis

Sensitivity and specificity at pre-specified cut-offs will be estimated using standard approaches for proportions. The diagnostic performance of each biomarker will be assessed using ROC curves. All patients with MPM (n=83-120) will be included and compared with AECs and a random sample of non-MPM cases. Due to cost constraints related to SOMAscan analyses 83 AECs and 83 non-MPM cases will be randomly selected. All AECs and 378 non-MPM cases will be used for Fibullin-3 analyses. Logistic regression will be used to estimate a diagnostic model using biomarker results. Cross validation will be used to provide robust estimates of AUC and specificity at fixed sensitivity rates of 80%, 90% and 95%.

Secondary Analysis

A prognostic model will be developed using Cox proportional hazard techniques. The modelling process will incorporate biomarker measurements (at presentation (both markers) and at 3 months (Fibulin-3 only) and other known prognostic features (e.g. performance status, histology).

Exploratory Analysis

The association between SOMAscan results/fibulin-3 in blood and tumour volume/measures of tumour angiogenesis will be estimated by Pearson or Spearman correlation, depending on the normality of the data. The same methods will be used to test the association between fibulin-3 in blood and pleural fluid. Changes in Fibulin-3 levels before and after histological sampling (at 1 month follow-up) will be compared using a paired t-test or Wilcoxon signed rank sum test (depending on the normality of the data).

**Changes to the Study Protocol since Trial Opening**

The protocol described accurately reflects Version 5, of the protocol, dated 17/6/16. The following changes were made in previous versions:

- Version 2, dated 14/2/14:
  - Safety reporting reduced following risk assessment by study Sponsor.
  - Collection of duplicate blood samples as provision for loss or damage and for sample retention in tissue bank.
  - Greater flexibility to timing of first blood draw.
- Version 3, dated 17/10/14:
  - Addition of recruitment of Controls from Respiratory Medicine clinics
  - Addition of exclusion criteria for patients with chest drains in-situ.
  - Eligibility for the MRI sub-study extended to patients proceeding to image-guided pleural biopsy
- Version 4, dated 27/4/15:
  - Update to the exclusion criteria for the AECs to include known or suspected thoracic malignancy under investigation.

- Version 5, dated 17/6/16:
  - Power projections adjusted based on interim reporting of MPM incidence from recruiting centres.

**Definition of End of Study**

The trial will end 2 years after the last patient with confirmed MPM is recruited or whenever all patients with MPM have died (whichever occurs first).

## ETHICS AND DISSEMINATION

### Ethics

The study protocol, all documents and amendments have been approved by the West of Scotland Research Ethics Service (Ref: 13/WS/0240).

### Monitoring, Data Management and Quality Assurance

No on-site monitoring will be undertaken. Two telephone-monitoring calls will be conducted by a CRUK Glasgow CTU Monitor to carry out process, compliance and documentation checks. Central monitoring of trial data will be performed by the Trial Statistician and Clinical Trial Co-ordinator by checking incoming forms for compliance with the protocol, data consistency, missing data and timing. The CRUK Glasgow CTU will control data consistency and data quality by entering trial data onto CTU database. Computerised and manual consistency checks will be performed and queries issued in cases of inconsistency or missing information. An audit trail of changes to the database will be maintained.

## Safety Considerations

Participants in the MRI sub-study will be asked at their 1-month follow-up visit about the occurrence of Adverse Events (AEs) related to the administration of MRI contrast (Gadolinium). These will be followed until resolution.

## Dissemination

The results of the study will be presented at national and international scientific meetings and published in full in a peer-reviewed journal (authorship will be according to that journal's guidelines). A lay summary will be produced and disseminated to interested parties.

## Trial Management

The trial will be coordinated from CRUK Glasgow CTU by the Trial Management Group (TMG), including the Chief Investigator, selected co-investigators, project manager, trial statistician, clinical trial co-ordinator and IT staff. The TMG will oversee the running of the trial and meet monthly.

## Acknowledgements

The authors are grateful to the Chief Scientist Office (Scotland) as the study funder, cancer research networks infrastructure, the participating sites and staff, SomaLogic Inc and the patients involved. ST is part funded by the West of Scotland Lung Cancer Research Fund. KGB is part funded by the NHS Career Research Fellowship. CR-UK and ECMC are acknowledged by FT and CMcC.

## REFERENCES


1 Musk AW, Olsen N, Alfonso H, *et al.* Predicting survival in malignant mesothelioma. *The European respiratory journal* 2011;**38**:1420–4. doi:10.1183/09031936.00000811

2 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung cancer* 2015;**88**:344–8. doi:10.1016/j.lungcan.2015.03.005

3 Tsim S, Dick C, Roberts F, *et al.* 76 Early experience of a regional mesothelioma MDT in the West of Scotland. *Lung cancer* 2014;**83**:S28–9. doi:10.1016/S0169-5002(14)70076-5

4 Renshaw AA, Dean BR, Antman KH, *et al.* The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 1997;**111**:106–9.

5 Scherpereel A, Astoul P, Baas P, *et al.* Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. The European respiratory journal. 2010;**35**:479–95. doi:10.1183/09031936.00063109

6 Boutin C, Rey F. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: Diagnosis. *Cancer* 1993;**72**:389–93.

7 Rusch VW, Giroux D. Do we need a revised staging system for malignant

pleural mesothelioma? Analysis of the IASLC database. *Ann Cardiothorac Surg* 2012;**1**:438–48. doi:10.3978/j.issn.2225-319X.2012.11.10

8 Salyer WR, Eggleston JC, Erozan YS. Efficacy of pleural needle biopsy and pleural fluid cytopathology in the diagnosis of malignant neoplasm involving the pleura. *Chest* 1975;**67**:536–9.

9 Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. *Mayo Clin Proc* 1985;**60**:158–64.

10 Nance KV, Shermer RW, Askin FB. Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid examination. *Mod Pathol* 1991;**4**:320–4.

11 O'Rourke N, Garcia JC, Paul J, *et al.* A randomised controlled trial of intervention site radiotherapy in malignant pleural mesothelioma. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* 2007;**84**:18–22. doi:10.1016/j.radonc.2007.05.022

12 Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. *Chest* 1995;**108**:754–8.

13 Robinson BWS, Creaney J, Lake R, *et al.* Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet* 2003;**362**:1612–6. doi:10.1016/S0140-6736(03)14794-0

14 Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax* 2014;**69**:895–902. doi:10.1136/thoraxjnl-2014-205205

15 Onda M, Nagata S, Ho M, *et al.* Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. *Clin Cancer Res* 2006;**12**:4225–31. doi:10.1158/1078-0432.CCR-06-0472

16 Creaney J, Yeoman D, Demelker Y, *et al.* Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. *J Thorac Oncol* 2008;**3**:851–7. doi:10.1097/JTO.0b013e318180477b

17 Pass HI, Lott D, Lonardo F, *et al.* Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *The New England journal of medicine* 2005;**353**:1564–73. doi:10.1056/NEJMoa051185

18 Chang K, Pai LH, Batra JK, *et al.* Characterization of the Antigen (Cak1) Recognized by Monoclonal-Antibody K1 Present on Ovarian Cancers and Normal Mesothelium. *Cancer research* 1992;**52**:181–6.

19 Chang K, Pai LH, Pass H, *et al.* Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg Pathol* 1992;**16**:259–68.

20 Hollevoet K, Nackaerts K, Thimpont J, *et al.* Diagnostic Performance of Soluble Mesothelin and Megakaryocyte Potentiating Factor in Mesothelioma. *Am J Respir Crit Care Med* 2010;**181**:620–5.

doi:10.1164/rccm.200907-1020OC

21 Hooper C, Lee YCG, Maskell N, *et al.* Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax. 2010;**65 Suppl 2**:ii4–17. doi:10.1136/thx.2010.136978

22 Hollevoet K, Nackaerts K, Thas O, *et al.* THe effect of clinical covariates on the diagnostic and prognostic value of soluble mesothelin and megakaryocyte potentiating factor. *Chest* 2012;**141**:477–84. doi:10.1378/chest.11-0129

23 Bergamini S, Bellei E, Reggiani Bonetti L, *et al.* Inflammation: an important parameter in the search of prostate cancer biomarkers. *Proteome science* 2014;**12**:32. doi:10.1186/1477-5956-12-32

24 Yuan JJ, Coplen DE, Petros JA, *et al.* Effects of rectal examination, prostatic massage, ultrasonography and needle biopsy on serum prostate specific antigen levels. *The Journal of urology* 1992;**147**:810–4.

25 Wong V, Wang D-Y, Warren K, *et al.* The effects of timing of fine needle aspiration biopsies on gene expression profiles in breast cancers. *BMC cancer* 2008;**8**:277. doi:10.1186/1471-2407-8-277

26 Pass HI, Levin SM, Harbut MR, *et al.* Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *The New England journal of medicine* 2012;**367**:1417–27. doi:10.1056/NEJMoa1115050

27 Kirschner MB, Pulford E, Hoda MA, *et al.* Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis.

*British journal of cancer* 2015;**113**:963–9. doi:10.1038/bjc.2015.286

28 Ostroff RM, Mehan MR, Stewart A, *et al.* Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. *PLoS ONE* 2012;**7**:e46091. doi:10.1371/journal.pone.0046091

29 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004. doi:10.1371/journal.pone.0015004

30 Kao SCH, Pavlakis N, Harvie R, *et al.* High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. *Clin Cancer Res* 2010;**16**:5805–13. doi:10.1158/1078-0432.CCR-10-2245

31 Hooper CE, Lyburn ID, Searle J, *et al.* The South West Area Mesothelioma and Pemetrexed trial: a multicentre prospective observational study evaluating novel markers of chemotherapy response and prognostication. *British journal of cancer* 2015;**112**:1175–82. doi:10.1038/bjc.2015.62

32 Pinato DJ, Mauri FA, Ramakrishnan R, *et al.* Inflammation-based prognostic indices in malignant pleural mesothelioma. *J Thorac Oncol* 2012;**7**:587–94. doi:10.1097/JTO.0b013e31823f45c1

33 Burt BM, Rodig SJ, Tilleman TR, *et al.* Circulating and tumor-infiltrating myeloid cells predict survival in human pleural mesothelioma. *Cancer* 2011;**117**:5234–44. doi:10.1002/cncr.26143

34 Zhang Y, Marmorstein LY. Focus on molecules: fibulin-3 (EFEMP1). *Exp Eye Res* 2010;**90**:374–5. doi:10.1016/j.exer.2009.09.018

35 Agha MA, El-Habashy MM, El-Shazly RA. Role of fibulin-3 in the diagnosis of malignant mesothelioma. *Egyptian Journal of Chest Diseases and Tuberculosis* 2014;**63**:99–105. doi:10.1016/j.ejcdt.2013.10.004

36 Corradi M, GOLDONI M, Alinovi R, *et al.* YKL-40 and mesothelin in the blood of patients with malignant mesothelioma, lung cancer and asbestosis. *Anticancer …* 2013.

37 Creaney J, Francis RJ, Dick IM, *et al.* Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden. *Clin Cancer Res* 2011;**17**:1181–9. doi:10.1158/1078-0432.CCR-10-1929

38 Pairon J-C, Laurent F, Rinaldo M, *et al.* Pleural plaques and the risk of pleural mesothelioma. *J Natl Cancer Inst* 2013;**105**:293–301. doi:10.1093/jnci/djs513

39 Paris C, Thierry S, Brochard P, *et al.* Pleural plaques and asbestosis: dose- and time-response relationships based on HRCT data. *The European respiratory journal* 2009;**34**:72–9. doi:10.1183/09031936.00094008

40 Baldwin DR, Callister MEJ, Guideline Development Group. The British Thoracic Society guidelines on the investigation and management of pulmonary nodules. *Thorax* 2015;**70**:794–8. doi:10.1136/thoraxjnl-2015-

207221

41 Administration UFAD. *Guidance for industry, bioanalytical methods validation*. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm 2013.

## CONTRIBUTIONS

Selina Tsim

- Contribution to the conception or design of the work; data acquisition, analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Caroline Kelly

- Contribution to the design of the work; analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Laura Alexander

- Contribution to the design of the work and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that

questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Carol McCormick

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Fiona Thomson

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Rosie Woodward

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content

- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

John E Foster

- Contribution to the conception and design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

David B Stobo

- Contribution to the design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Jim Paul

- Contribution to the conception and design of the work; data analysis

or interpretation of data for the work

- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Nick A Maskell

- Contribution to the design of the work; data analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Anthony Chalmers

- Contribution to the conception and design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kevin G Blyth

- Principal contribution to the conception and design of the work; data acquisition, analysis and interpretation of data for the work
- Drafting the work
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

**FUNDING**

This work was supported by the Chief Scientist's Office of the Scottish Government (Project Grant ETM/285) and the West of Scotland Lung Cancer Research Group (Award September 2015). KGB is part-funded by NHS Research Scotland.

**COMPETING INTERESTS STATEMENT**

SomaLogic Inc. have provided funding for all SOMAscan assays.

## FIGURE LEGENDS

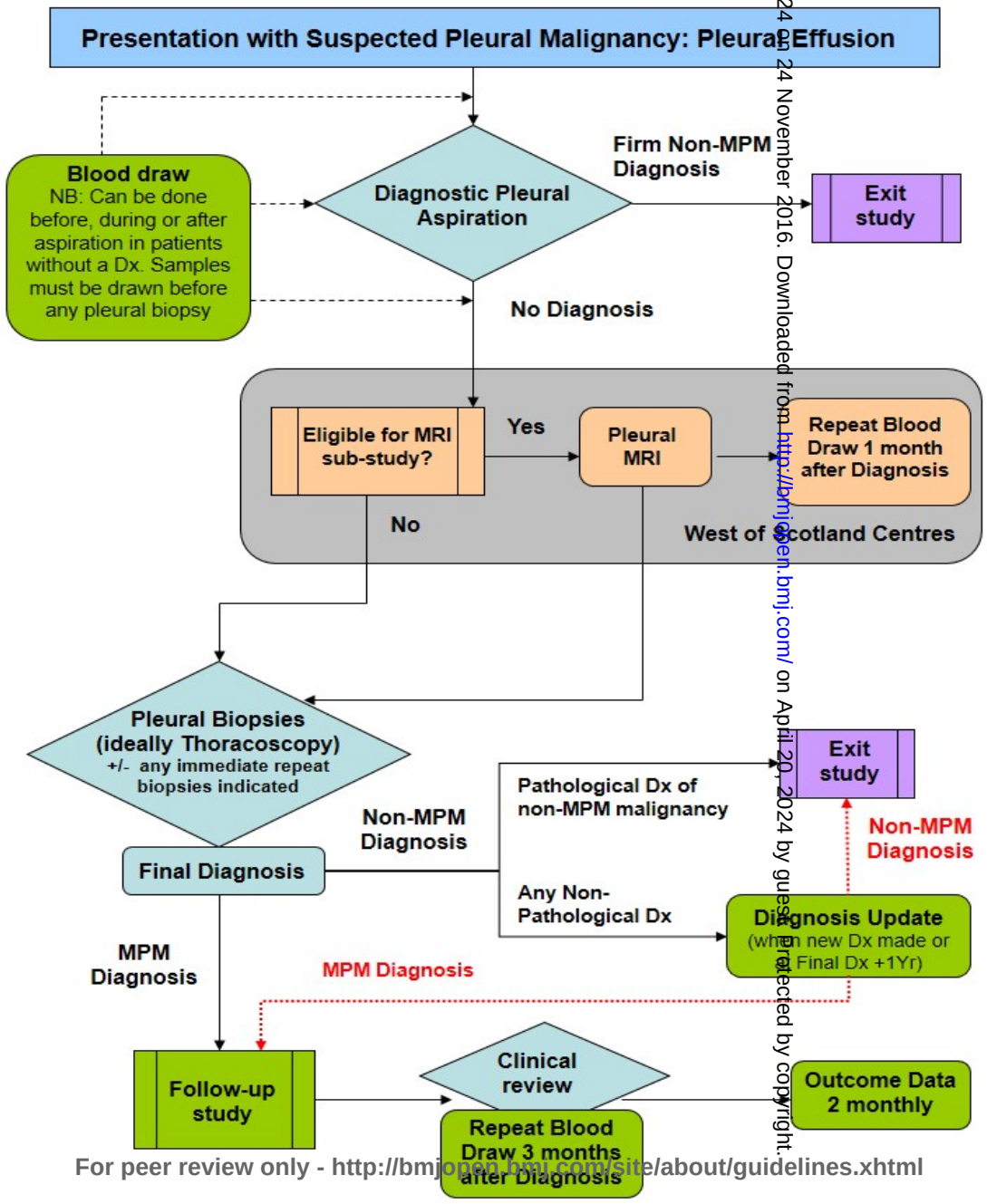
### Figure 1

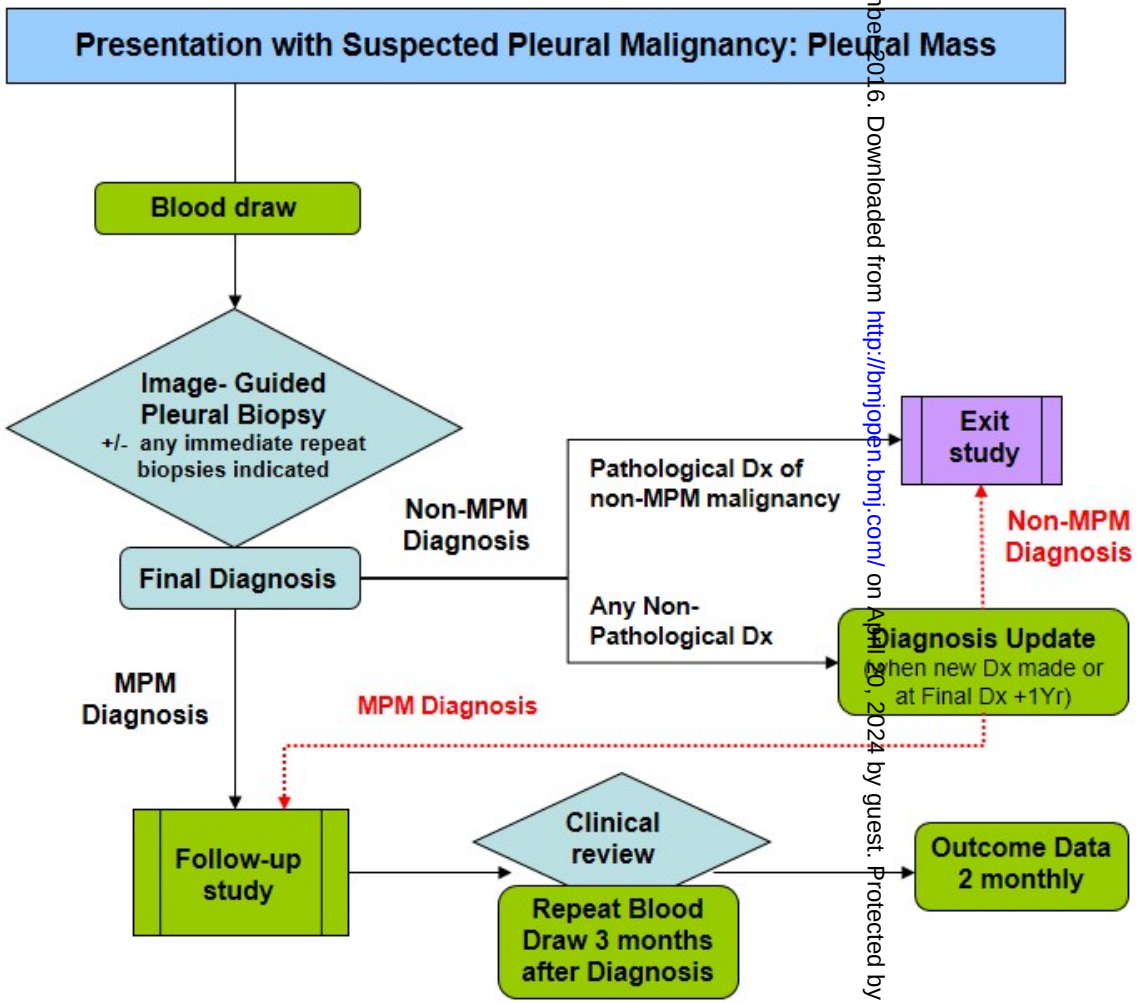
Summary of the design of the DIAPHRAGM study. Figure 1(a) relates to patients with Pleural Effusion and Figure 1(b) relates to patients with a Pleural Mass, but no significant fluid component.

# TABLES

## Table 1. Outcome Measures used in the DIAPHRAGM study

| Research Objective | Outcome Measures |
|---|---|
| **Primary** | |
| To determine whether SOMAscan results and/or Fibulin-3 levels in blood at presentation can differentiate MPM from asbestos-exposed controls and patients with other causes of pleural effusion with a sufficient degree of sensitivity and specificity to be of routine clinical value | Serum SOMAscan<br>Plasma Fibulin-3<br>Final diagnosis reached |
| **Secondary** | |
| To determine whether:<br>1. SOMAscan results and/or Fibulin-3 levels at presentation provide clinically useful prognostic information in MPM patients | Serum SOMAscan & plasma Fibulin-3 at presentation<br>Survival (from registration) |
| 2. early changes in Fibulin-3 levels after diagnosis (at 3 months) are associated with a poorer prognosis in MPM | Plasma Fibulin-3 3 months post-Dx<br>Survival (from registration) |
| **Exploratory** | |
| To determine whether:<br>1. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour volume, defined by MRI | Serum SOMAscan<br>Plasma Fibulin-3<br>MPM tumour volume at MRI, defined using Myrian Intrasense™ software |
| 2. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour angiogenesis (as defined by perfusion-based MRI biomarkers) | Serum SOMAscan<br>Plasma Fibulin-3<br>The following MRI biomarkers:<br>• MRI-ECE<br>• Redistribution rate constant ($K_{ep}$)<br>• Elimination rate constant ($K_{el}$)) |
| 3. there is a correlation between Fibulin-3 levels in blood and pleural fluid at presentation in patients with MPM | Fibulin-3 in paired blood and pleural fluid samples |
| 4. Fibulin-3 results are affected by pleural biopsy +/- fluid drainage and pleurodesis at the time of diagnosis | Fibulin-3 at presentation and at 1 month post-biopsy +/- drainage and pleurodesis |






SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description |
|---|---|---|
| **Administrative information** | | |
| √Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym |
| √Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry |
| | 2b | All items from the World Health Organization Trial Registration Data Set |
| √Protocol version | 3 | Date and version identifier |
| √Funding | 4 | Sources and types of financial, material, and other support |
| √Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors |
| | 5b | Name and contact information for the trial sponsor |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) |
| **Introduction** | | |
| √Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention |
| | 6b | Explanation for choice of comparators |
| √Objectives | 7 | Specific objectives or hypotheses |
| √Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) |

**Methods: Participants, interventions, and outcomes**

| | | |
|---|---|---|
| √Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained |
| √Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) |
| √Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial |
| √Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended |
| √Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) |
| √Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations |
| √Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size |

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

| | | |
|---|---|---|
| NA Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions |

| | | |
|---|---|---|
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned |
| NA Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions |
| NA Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial |
| **Methods: Data collection, management, and analysis** | | |
| √Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols |
| √Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol |
| √Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) |
| **Methods: Monitoring** | | |
| √Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed |

| | | |
|---|---|---|
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial |
| √Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct |
| √Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor |
| **Ethics and dissemination** | | |
| √Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval |
| √Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) |
| √Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable |
| √Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial |
| √Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site |
| √Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators |
| √Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation |
| √Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code |

## Appendices

| | | |
|---|---|---|
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

# Diagnostic and prognostic biomarkers in the rational assessment of mesothelioma (DIAPHRAGM) study: protocol of a prospective, multi-centre, observational study



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2016-013324.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 13-Sep-2016 |
| Complete List of Authors: | Tsim, Selina; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Cancer Sciences<br>Kelly, Caroline; Cancer Research UK Glasgow Clinical Trials Unit<br>Alexander, Laura; Cancer Research UK Glasgow Clinical Trials Unit<br>McCormick, Carol; Cancer Research UK Glasgow Clinical Trials Unit<br>Thomson, Fiona; Cancer Research UK Glasgow Clinical Trials Unit<br>Woodward, Rosemary; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility<br>Foster, John; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility<br>Stobo, David; Queen Elizabeth University Hospital, Radiology<br>Paul, Jim; Cancer Research UK Glasgow Clinical Trials Unit<br>Maskell, Nick; University of Bristol, Academic Respiratory Unit, School of Clinical Sciences; North Bristol NHS Trust, Respiratory Research Unit<br>Chalmers, Anthony; University of Glasgow, Institute of Cancer Sciences; Beatson West of Scotland Cancer Centre<br>Blyth, Kevin; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Infection, Immunity and Inflammation |
| Primary Subject Heading: | Respiratory medicine |
| Secondary Subject Heading: | Oncology, Patient-centred medicine, Research methods |
| Keywords: | Mesothelioma, Biomarker, Diagnosis, Prognosis |
| | |

**TITLE:**

# DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN THE RATIONAL ASSESSMENT OF MESOTHELIOMA (DIAPHRAGM) STUDY: PROTOCOL OF A PROSPECTIVE, MULTI-CENTRE, OBSERVATIONAL STUDY

**AUTHORS:**

Selina Tsim[1/2], Caroline Kelly[3], Laura Alexander[3], Carol McCormick[3], Fiona Thomson[3], Rosie Woodward[4], John E Foster[4], David B Stobo[5], Jim Paul[3], Nick A Maskell[6], Anthony Chalmers[2/7], Kevin G Blyth[1/8]

**Affiliations**

1. Department of Respiratory Medicine, Queen Elizabeth University Hospital, Glasgow, U.K.
2. Institute of Cancer Sciences, University of Glasgow, U.K.
3. Cancer Research UK Glasgow Clinical Trials Unit, UK
4. Glasgow Clinical Research Imaging Facility, Queen Elizabeth University Hospital, Glasgow, U.K.
5. Department of Radiology, Queen Elizabeth University Hospital, Glasgow, U.K.
6. Academic Respiratory Unit, School of Clinical Sciences, University of Bristol, UK
7. Beatson West of Scotland Cancer Centre, Glasgow, UK
8. Institute of Infection, Immunology and Inflammation, University of Glasgow, U.K.

**Corresponding Author and Address**

Kevin G. Blyth

Consultant Respiratory Physician and NRS Career Research Fellow

Department of Respiratory Medicine, Administration Building,

Queen Elizabeth University Hospital,

Glasgow, G51 4TF

kevin.blyth@ggc.scot.nhs.uk

Tel: +44 141 451 6099

**Keywords:**

Mesothelioma

Biomarker

Diagnosis

Prognosis

**WORD COUNT:**

3998/4000

## ABSTRACT (300/300)

### INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer, which is difficult to diagnose. Thoracoscopy is frequently required but is not widely available. An accurate, non-invasive diagnostic biomarker would allow early specialist referral, limit diagnostic delays and maximize clinical trial access. Current markers offer insufficient sensitivity and are not routinely used. The SOMAmer® proteomic classifier and Fibulin-3 have recently demonstrated sensitivity and specificity exceeding 90% in retrospective studies. DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma) is a suitably powered, multi-centre, prospective observational study designed to determine whether these markers provide clinically useful diagnostic and prognostic information.

### METHODS AND ANALYSIS

Serum and plasma (for SOMAscan® and Fibulin-3, respectively) will be collected at presentation, prior to pleural biopsy/pleurodesis, from 83-120 MPM patients, 634-724 patients with non-MPM pleural disease and 109 asbestos-exposed controls. Final numbers of MPM/non-MPM cases will depend on the incidence of MPM in the study population (estimated at 13-20%). Identical sampling and storage protocols will be used in 22 recruiting centres and histological confirmation sought in all cases. Markers will be measured using the SOMAscan proteomic assay (SomaLogic Inc.) and a commercially available Fibulin-3 ELISA (USCN Life Science Inc.). The standard error in the estimated sensitivity and specificity will be <5% for each

marker and their performance will be compared to serum Mesothelin. Blood levels will be compared to paired pleural fluid levels and MPM tumour volume (using Magnetic Resonance Imaging) in a nested sub-study. The prognostic value of each marker will be assessed and a large bioresource created.

## ETHICS AND DISSEMINATION

The study has been approved by the West of Scotland Research Ethics Committee (Ref: 13/WS/0240). A Trial Management Group meets on a monthly basis. Results will be published in peer-reviewed journals, presented at international meetings and disseminated to patient groups.

**TRIAL REGISTRATION NUMBER:** ISRCTN10079972

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- Prospective, multi-centre study recruiting a representative sample of patients in an intention-to-diagnose population
- Strict sampling, processing and storage methods used in all patients
- Robust diagnostics and 12 months' follow-up
- Creation of a large bio-resource annotated with detailed, prospectively collected clinical information, for use in future biomarker discovery and validation studies
- The final number of study participants with MPM, and therefore the power available to test the primary objective, will not be known until recruitment is complete.

## INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an invasive thoracic malignancy, strongly associated with prior asbestos exposure. The median survival for patients with MPM is poor at 9-10 months [1,2]. However, the prognosis of individuals is highly variable and largely determined by histological subtype [2]. MPM frequently presents as an emergency with a large, symptomatic pleural effusion [3]. Early specialist referral is frequently required because pleural fluid aspiration cytology is unreliable [4] and histological confirmation is recommended in all patients [5]. Thoracoscopy (under local or general anaesthesia) [5], enables widespread tissue sampling [6] with diagnostic yields for malignancy >90% [7] but is not available in all centres. Thoracoscopy also allows pleurodesis or indwelling pleural catheter placement.

A reliable, non-invasive diagnostic biomarker for MPM would be a major clinical advance. This would allow clinicians to reliably differentiate likely MPM from secondary pleural malignancies (e.g. lung or breast cancer), which may present with similar clinical and imaging features but require less evolved diagnostic pathways. This reflects the improved sensitivity of pleural cytology in these diseases [8-10] and the frequent option of alternative sites for tissue biopsy. A positive MPM biomarker test could facilitate early referral to a thoracoscopy centre and avoid unnecessary diagnostic delay (e.g. due to repeated pleural aspirations), minimising the risk of subsequent needle-tract metastases [11,12] and maximizing opportunity for clinical trial enrolment. Previous studies have demonstrated that blood levels of single proteins,

including mesothelin [13,14], megakaryocyte potentiating factor (MPF) [15,16] and osteopontin [17], are higher in patients with MPM than in asbestos-exposed controls (AECs) and patients with secondary pleural malignancies. Mesothelin, a cell-adhesion glycoprotein that is over-expressed in MPM [18,19] is the most widely studied and is associated with an MPM sensitivity of 56-77% at 95% specificity [14,16,20]. However, a recent meta-analysis (of 4491 individuals (1026 with MPM)) reported a sensitivity of only 32% at 95% specificity. Mesothelin does not, therefore, contribute to current diagnostic algorithms [21]. MPF offers no advantage over mesothelin [16], while the clinical utility of osteopontin is limited by stability and reproducibility concerns [17].

An ideal MPM biomarker would be measurable in blood for ease of collection and offer sufficient sensitivity at high specificity in patients presenting with suspected MPM. Differentiation between advanced disease patients and appropriate controls is of limited value. High specificity is mandatory for a low prevalence disease, and should apply to patients with asbestos exposure and non-MPM pleural disease. Biomarker results should also correlate with disease extent and have defined relationships with potential confounders including renal function [22] and the effect of pleural interventions. The latter is important because the precedent has been established in prostate [23,24] and breast cancer [25], that recent sampling, resection or peri-tumoural inflammation may affect biomarker expression. This is particularly relevant to MPM where biopsies are frequently large and often combined with pleurodesis. Several previous biomarker studies, which validated

inconsistently in external populations, used samples acquired at later time-points, often post-diagnosis (and post-pleurodesis) including samples taken prior to, during, or after resection surgery [17,26,27]. The aim of the DIAPHRAGM study is to prospectively evaluate the diagnostic and prognostic performance of the SOMAscan proteomic classifier [28] and fibulin-3 [26], which have demonstrated high sensitivity and specificity in recent retrospective series. The study has been designed to generate clinically meaningful results, which can be related to MPM biology and confounding factors, and applied to patients at first presentation.

**SOMAmer-based Proteomic Classifier**

The SOMAscan assay is a highly multiplexed proteomic platform that utilizes SOMAmer (Slow Off-rate Modified Aptamers) reagents to selectively bind and quantify proteins [29]. A 13-protein classifier was developed by SomaLogic Inc. (Boulder, Colorado), using this novel proteomics-based biomarker detection technique [28] in a retrospective study over 800 proteins were measured in the serum of 117 MPM patients and 142 AECs, collected at surgical MPM centres in the US between 1996 and 2011. Using a panel of 13 differentially expressed proteins and a cut-point of 0.5, the classifier was able to segregate MPM from controls with an area under the curve (AUC) of 0.99 +/- 0.01 in training (60 MPM/60 controls), 0.98 +/- 0.04 in blinded verification (19 MPM/20 controls) and 0.95 +/- 0.04 in blinded validation sets (38 cases/62 controls) [28]. The combined sensitivity for the three cohorts was 93% at 91% specificity. Based on the published ROC curve for the validation cohort, sensitivity at 95% specificity appeared to be approximately 78%,

although the authors did not report this value. This performance exceeds that of any previous MPM biomarker, although the classifier's specificity appeared lower in patients with non-MPM pleural effusion (n=32). There was a modest correlation between classifier score and disease stage, but prognostic significance was not assessed. The 13 classifier proteins (nine up-regulated, four down-regulated) have not previously been associated with MPM. Their functions fall into two broad groups; regulation of proliferation and inflammation. Quite apart from their biological relevance to MPM, the latter is an important potential confounder because many of the patients involved will have previously undergone pleurodesis. In addition, several groups have reported an independent interaction between prognosis and inflammatory biomarkers in MPM, including neutrophil-to-lymphocyte ratio (NLR) [30-32], monocytosis [33] and the modified Glasgow Prognostic Score [32]. Therefore, adequate understanding of the diagnostic and prognostic utility of this assay requires replication in a pre-pleurodesis cohort and prospective evaluation of interactions between inflammatory biomarkers and SOMAscan scores.

**Fibulin-3**

Fibulin-3 is a secreted glycoprotein, encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [34]. Fibulin-3 is over-expressed in MPM tumours relative to adjacent benign pleura [26] and expressed and secreted by MPM cell lines [27]. Pass et al retrospectively measured fibulin-3 in the plasma of 92 MPM patients, 136 AECs, 93 patients with non-MPM pleural effusion and 43 healthy controls [26].

A plasma cut-point of 52 ng/ml provided 97% sensitivity at 95% specificity and a 95% CI of the AUC of 0.97-0.99 in differentiating MPM from all other cases. However, in a blinded external validation set, sensitivity was below 40% (at 95% specificity), with an AUC=0.87.

Subsequent studies have revealed mixed results. In a study of 153 patients (82 with MPM), Creaney et al reported a sensitivity of 22% (at 95% specificity) at the same 52 ng/ml cut-point and an AUC of 0.671 (0.606 to 0.732), which was significantly inferior to mesothelin measured in the same patients (sensitivity 56% (at 95% specificity); AUC 0.816 (0.755 to 0.867)) at a 2.5 nM threshold [14]). In a small Egyptian study using an unspecified Fibulin-3 assay and internally-defined cut-points, Agha et al reported 100% sensitivity/78% specificity in differentiating MPM cases (n=25) from non-malignant pleural disease (n=9), and 88% sensitivity/82% specificity in differentiating MPM from secondary pleural malignancies (n=11) [35]. No combined sensitivity was reported. An Italian study found no difference in Fibulin-3 levels but used serum (not plasma), a control group without pleural disease (Asbestosis) and contained only 14 patients with MPM [36].

## METHODS AND ANALYSIS

### Study Design

DIAPHRAGM is a prospective, multi-centre observational study. The study incorporates sampling windows that correspond to the proposed use of a diagnostic biomarker, i.e. at presentation with Suspected Pleural Malignancy (SPM). The overall study design is summarized in Figure 1(a) and 1(b). The

main impact of this design is that biomarkers will be drawn before a diagnosis is made. In addition to better replicating the future use of these markers, this avoids the potential confounding effect of pleurodesis on biomarker results. The diagnostic performance of the SOMAmer panel and Fibulin-3 will be assessed using cut-points determined in the relevant original studies and compared to mesothelin (using the MESOMARK® ELISA (Fujirebio Diagnostics Inc, PA, USA). Identical processing and storage protocols will be used in patients with SPM and a group of AECs. Potential confounders including renal function, inflammatory indices and drugs will be recorded at all visits. The timing of the biomarker blood draw in relation to pleural aspiration (pre-aspiration or post-aspiration) will be recorded in order to assess the effect of this intervention on biomarker results. An exploratory, cross-sectional Magnetic Resonance Imaging (MRI) sub-study will determine if there is any correlation between blood biomarker levels and MPM tumour volume, as has been established for Mesothelin using Computed Tomography-Positron Emission Tomography scanning [37].

**Study Objectives and Outcome Measures**

These are presented in Table 1.

**Setting**

At least 600 consecutive patients with SPM will be recruited from 21 centres (20 in the UK, 1 in Republic of Ireland). These are a mixture of academic and more clinically orientated units. This should make the results of the DIAPHRAGM study generalizable to patients presenting with SPM to acute

hospital services. The principal criterion used to select centres was that they had sufficiently evolved pleural diagnostic services to deliver a reliable diagnosis. Specifically, access to on-site thoracoscopy (ideally including local anaesthetic thoracoscopy (LAT)) and a regional mesothelioma MDT meeting (for diagnostic review and staging) was required.

**Screening and Eligibility Assessment**

Suspected Pleural Malignancy

Cases will be identified on presentation to a Respiratory out-patient clinic or acute hospital admissions unit. This will be based on the history, examination and available investigations. Potentially eligible patients will be provided with the study Patient Information Sheet (PIS, see Online Supplementary Appendix 1) and eligibility assessed based on the following criteria:

*Inclusion Criteria:*

- SPM, defined by a unilateral pleural effusion or pleural mass lesion
- Sufficient fitness for diagnostic sampling (site investigator's clinical judgment)
- Informed written consent

*Exclusion Criteria:*

- Intercostal chest drain in-situ, or inserted within the previous 3 months

Asbestos-related pleural plaques are not an inclusion criterion since these are absent in up to 25% of MPM cases [38], and are also common in asbestos-

exposed populations without MPM [39]. Patients with lung nodules or other visceral mass lesions are not excluded, assuming the investigator suspects pleural malignancy. This is because of the high prevalence of lung nodules in the target population (older patients, commonly smokers) and the high false positive rate of CT imaging in this regard [40].

Subjects recruited to the SPM arm will generate cohorts of MPM and non-MPM pleural disease of various aetiologies, likely including Benign Asbestos-related Pleural Effusion and secondary pleural malignancies. These numbers will be sufficient to address the primary objective with sufficient statistical power to inform clinical practice (see later section).

Asbestos-exposed control (AEC) subjects

109 AECs will be recruited via invitations sent by Clydeside Action on Asbestos (CAA), an advocacy body based in Glasgow with a database of over 600 clients, or by Respiratory clinics at the host centre. Individuals will be invited to participate by letter (if identified via CAA) or given the PIS (see Online Supplementary Appendix 2) at clinic. All subjects will be invited to a single research clinic visit assuming the following Eligibility Criteria are met.

*Inclusion Criteria:*

- Documented history of asbestos exposure and associated pleural plaques, asbestosis or diffuse pleural thickening
- Willing and able to travel to a research clinic interview in Glasgow
- Informed written consent

*Exclusion Criteria:*

- Known MPM
- Known or suspected other thoracic malignancy under investigation
- Known pleural effusion of any cause

Detailed asbestos exposure histories will be taken from all participants in both the suspected pleural malignancy cohort and the asbestos-exposed control cohort. This will be done using an asbestos exposure questionnaire derived from Health and Safety Executive asbestos survey [27] (see Online Supplementary Appendix 3). This questionnaire includes recording of the nature of occupational exposure(s), which can be correlated to likely fibre exposure. The duration and first year of exposure is also recorded. Non-occupational sources of exposure are also recorded (e.g. the washing of an occupationally exposed spouse's work clothes). Only AECs with documented imaging sequelae of asbestos exposure (e.g. pleural plaques) and an asbestos exposure history will be included.

Cross-sectional MRI sub-study

50 patients will be recruited to address the study's exploratory objectives (see Table 1). Eligibility will be determined based on the following criteria.

*Inclusion Criteria:*

- Pleural histological sampling (by LAT/image-guided biopsy) indicated to investigate SPM following a non-diagnostic pleural aspiration
- Recruited in a West of Scotland centre

*Exclusion Criteria:*

- Unable to undergo MRI (claustrophobia or known contraindications such as pacemaker, ferrous metal implants or foreign body)
- Allergy to Gadolinium contrast
- Renal impairment (eGFR <30ml/min)
- Pregnancy

Based on previous audit data from the host centre we expect at least 40% (n=20) of patients in the sub-study to have MPM. Eligible subjects will be approached at the clinical visit during which non-diagnostic pleural aspiration results, and the need for further investigation, are discussed. Subjects will be provided with a separate PIS (see Online Supplementary Appendix 4) and will be asked to provide additional informed written consent.

**Consent**

All subjects will be given sufficient time (as judged by themselves) to provide written informed consent after reading the relevant PIS and having the opportunity to ask questions.

**Outcome Measures**

The outcome measures associated with each of the trial's objectives are detailed in Table 1.

*Final Diagnosis*

A specific cytological or histological pleural diagnosis will be sought in all patients according to national guidelines [21]. This will be recorded as the

Final Diagnosis, which may be based on immediate repeat biopsies felt to be indicated by the site PI (see Figure 1). Any cytologically or histologically confirmed non-MPM diagnosis (e.g. pleural metastases from lung cancer) will be recorded without the need for any further updates. However, sites will need to provide updates for any non-MPM diagnosis that is not cytologically or histologically confirmed (e.g. parapneumonic effusion). These will be submitted on the 12-month anniversary of the original diagnosis, or as soon as any new pleural diagnosis is made. This aims to capture any false negative diagnostic tests from the initial presentation, acknowledging the major diagnostic challenges posed by pleural malignancies, particularly MPM.

*Biomarker Sampling and Storage*

Blood samples (+/- pleural fluid in WoS centres) will be drawn and immediate processing performed at each study centre. Samples can be taken before or after pleural aspiration. Patients with positive pleural cytology cannot be recruited (see Figure 1(a)). Duplicate samples will be collected for all measurements at all visits, ensuring redundancy in case of loss or damage to samples during transportation to the appropriate central laboratory. SOMAmer biomarker levels will be measured in serum; therefore, 9 ml of venous blood will be collected first into a vacutainer tube containing SST clot activator. Fibulin-3 levels will be measured in plasma; therefore, 9 ml of venous blood will be collected second into a vacutainer tube containing EDTA. In centres contributing to the exploratory MRI sub-study (WoS sties only) 20 ml of pleural fluid will be also collected into a plain container if pleural fluid is being drawn for diagnostic/therapeutic purposes at the same visit. If

not done at this first opportunity, pre-diagnosis pleural fluid can also be collected during local anaesthetic or general anaesthetic thoracoscopy, prior to any biopsy or pleurodesis being performed.

*Biomarker Processing and Storage*

Serum samples will be allowed to clot for 30 minutes before centrifugation. Plasma and pleural fluid samples will be centrifuged immediately. All samples will be centrifuged at 2200g for 15 minutes at room temperature. For all samples, the supernatant will be withdrawn by pipette, aliquoted into cryovials of at least 250µL volume, labeled and placed into a -80 freezer within 2 hours. Samples will be stored at each recruiting centre until batched transport to the appropriate study laboratory. Samples from WoS recruiting centres will be used to create a bioresource. The bioresource will be stored as a satellite collection of the NHS Greater Glasgow & Clyde Biorepository, a Health Improvement Scotland (HIS)-approved tissue bank. Data will be stored in the secure Cancer Research UK Clinical Trials Unit database. On study completion, investigators will be invited to apply for access to data and samples appropriate to their research questions. Access will be granted after peer review of each proposal by a scientific board comprising members of the DIAPHRAGM TMG and senior Biorepository staff. An annual update on this activity will be submitted to the West of Scotland Research Ethics Committee.

*Biomarker Analyses*

SomaLogic Inc. (Boulder, Colorado, USA) will perform all SOMAscan proteomic analyses [28]. This utlises SOMAmer reagents to specifically bind

to protein targets in blood. Relative protein concentrations will be converted to measurable nucleic acid signals that are quantified by hybridization to DNA microarrays [29].

Fibulin-3 and mesothelin levels will be measured by the Translational Pharmacology Unit, Wolfson Wohl Cancer Research Centre, UK, using ELISA methods validated according to the FDA-recommended guidelines for bioanalytical methods [41]. Fibulin-3 levels in plasma and pleural fluid will be measured using the commercially available ELISA (Cloud-Clone Corp., formerly USCN Life Science Inc, Houston, Texas, USA) as in the original Pass study [26]. Mesothelin will be measured using the MESOMARK® ELISA (Fujirebio Diagnostics, Inc, PA, USA). In parallel, we aim to develop a custom multiplex ELISA assay that has the potential to simultaneously measure multiple biomarkers (fibulin-3, mesothelin and osteopontin) with greater accuracy (U-PLEX, Meso Scale Diagnostics, Rockville, USA)

*Magnetic Resonance Imaging*

Patients will be scanned at the Queen Elizabeth University Hospital, Glasgow, on a 3.0T Siemens Verio MRI Scanner. After localisation of the affected thoracic cavity, an isotropic 3D T1-weighted volume will be acquired using VIBE sequences. A stack of axial slices covering the entire lung and surrounding pleura will be acquired as a set of short breath-holds. Gd-DTPA contrast (Gadovist) will be administered via a peripheral intravenous line as a 15-40 ml bolus (0.05 mmol/kg). VIBE sequences will be reacquired at copied slice positions to provide pre and post-contrast images. The total scan time

will be around 45 minutes. Regions of enhancing pleural tumour will be defined using semi-automated signal intensity thresholding based on contrast-enhanced axial slices using Myrian Intrasense™ software, which has previously been used to assess tumour volume in MPM. [42] MRI volumetry analyses will be validated using imaging phantoms.

*Survival*

Survival will be recorded in days from the date of study registration to the data of death, from any cause.

**Sample Size, Assumptions and Uncertainties**

Sample size estimations for each marker were based on published data at the point of study design and a projected MPM incidence of 13-20% in the SPM cohort. The power available to test the hypotheses below is therefore reported as a range, based on final MPM numbers lying between 83 (13% incidence) and 120 (20% incidence).

Primary Objective

*SOMAscan Assay*

We hypothesize that the MPM sensitivity and specificity exceed 90%, based on previously reported performance in combined training, verification and validation sets (sensitivity 93.2% (88.6–97.7%), specificity (90.8% (86.1–95.6%) [28]). Recruitment of 83-120 MPM patients will allow us to distinguish a sensitivity of >90% from a sensitivity <80% with 80-93% power, respectively, at the 5% 1-sided level of significance. 83-120 MPM patients will

allow discrimination between a specificity <80% and a specificity >90%, with 80-88% power at the 5% 1-sided level of statistical significance. The standard error in the estimated sensitivity and specificity will be less than 5%, across all possible outcomes.

*Fibulin-3*

We hypothesize that the MPM sensitivity will exceed 80% and that the specificity will exceed 90% (at the 52 ng/ml cutoff). These figures are based on a reduced level of performance to the primary results reported by Pass et al (97% sensitivity, 95% specificity), given lower sensitivity in the external validation cohort studied (40% at 95% specificity) [26].

With 83-120 MPM patients the study will be able to distinguish a sensitivity of >80% from a sensitivity <70% with 65-80% power, respectively, at the 5% 1-sided level of statistical significance. The standard error in the estimated sensitivity will be less than 5%. In order to achieve 90% power to distinguish a specificity of >90% from a specificity <85% at the 5% 1-sided level of statistical significance, a random sample of 378 non-MPM samples will be analysed. The standard error in the estimated specificity will be <2.3%.

The study data will be used to estimate the AUC for the SOMAscan marker for distinguishing MPM from non-MPM patients in the SPM cohort. Assuming 83-120 patients in the MPM group and 83-120 in the non-MPM group the AUC can be estimated with a 95% confidence interval of width 0.120-0.168 (assuming a cut-point exists with a reasonable sensitivity of 80% and a

modest specificity of 40%). If more sensitive/specific cut-points exist the width of the 95% confidence interval will be much reduced. The study data will be used to develop a new diagnostic signature based on Fibulin-3 and SOMAscan results to distinguish MPM from non-MPM effusions.

Secondary Objectives

The study data will be used to determine whether baseline SOMAscan results and/or fibulin-3 levels, or a change in levels at 3 months (Fibulin-3 only), are independent prognostic factors for MPM. A correlation of 0.4 between existing prognostic factors and each marker has been assumed. For the baseline levels, to detect an approximate doubling in median OS (from 6 month to 12 months - a hazard ratio of 2) with 80% power and 5% 2-sided level of statistical significance between a good/poor prognostic group based on dichotomising these markers requires at least 83 MPM patients recruited over three years with approximately 6 months subsequent follow-up to observe 66 deaths. For the 3-month change levels, a hazard ratio of 2.38 can be detected (80% power, 5% 2-sided level of statistical significance) when 49 deaths are observed in the estimated 66 out of 83 patients who survive to 3 months.

Exploratory Objectives

These will be addressed in the MRI sub-study, which will generate a sample of at least 20 MPM patients. This will allow moderately large associations (0.6) between the exploratory outcome measures (see Table 1) to be detected at 80% power at the 5%, two-sided level of statistical significance. The effect

of pleural biopsies +/- drainage/pleurodesis on Fibulin-3 levels will be assessed using all 50 patients recruited. This will allow moderately small differences (standardised difference of 0.4) to be detected with 80% power at the 5% two-sided level of statistical significance.

**Statistical Analysis Plan**

Primary Analysis

Sensitivity and specificity at pre-specified cut-offs will be estimated using standard approaches for proportions. The diagnostic performance of each biomarker will be assessed using ROC curves. All patients with MPM (n=83-120) will be included and compared with AECs and a random sample of non-MPM cases. Due to cost constraints related to SOMAscan analyses 83 AECs and 83 non-MPM cases will be randomly selected. All AECs and 378 non-MPM cases will be used for Fibullin-3 analyses. Logistic regression will be used to estimate a diagnostic model using biomarker results. Cross validation will be used to provide robust estimates of AUC and specificity at fixed sensitivity rates of 80%, 90% and 95%.

Secondary Analysis

A prognostic model will be developed using Cox proportional hazard techniques. The modelling process will incorporate biomarker measurements (at presentation (both markers) and at 3 months (Fibulin-3 only) and other known prognostic features (e.g. performance status, histology).

Exploratory Analysis

The association between SOMAscan results/fibulin-3 in blood and tumour volume/measures of tumour angiogenesis will be estimated by Pearson or Spearman correlation, depending on the normality of the data. The same methods will be used to test the association between fibulin-3 in blood and pleural fluid. Changes in Fibulin-3 levels before and after histological sampling (at 1 month follow-up) will be compared using a paired t-test or Wilcoxon signed rank sum test (depending on the normality of the data). Due to cost constraints, exploratory end-points involving pleural fluid SOMAscan results will be analysed at a later date.

**Changes to the Study Protocol since Trial Opening**

The protocol described accurately reflects Version 5, of the protocol, dated 17/6/16. The following changes were made in previous versions:

- Version 2, dated 14/2/14:
  - Safety reporting reduced following risk assessment by study Sponsor.
  - Collection of duplicate blood samples as provision for loss or damage and for sample retention in tissue bank.
  - Greater flexibility to timing of first blood draw.
- Version 3, dated 17/10/14:
  - Addition of recruitment of Controls from Respiratory Medicine clinics
  - Addition of exclusion criteria for patients with chest drains in-situ.
  - Eligibility for the MRI sub-study extended to patients proceeding to image-guided pleural biopsy
- Version 4, dated 27/4/15:

- Update to the exclusion criteria for the AECs to include known or suspected thoracic malignancy under investigation.

- Version 5, dated 17/6/16:
  - Power projections adjusted based on interim reporting of MPM incidence from recruiting centres.

**Definition of End of Study**

The trial will end 2 years after the last patient with confirmed MPM is recruited or whenever all patients with MPM have died (whichever occurs first).

## ETHICS AND DISSEMINATION

### Ethics

The study protocol, all documents and amendments have been approved by the West of Scotland Research Ethics Service (Ref: 13/WS/0240).

### Monitoring, Data Management and Quality Assurance

No on-site monitoring will be undertaken. Two telephone-monitoring calls will be conducted by a CRUK Glasgow CTU Monitor to carry out process, compliance and documentation checks. Central monitoring of trial data will be performed by the Trial Statistician and Clinical Trial Co-ordinator by checking incoming forms for compliance with the protocol, data consistency, missing data and timing. The CRUK Glasgow CTU will control data consistency and data quality by entering trial data onto CTU database. Computerised and manual consistency checks will be performed and queries issued in cases of

inconsistency or missing information. An audit trail of changes to the database will be maintained.

## Safety Considerations

Participants in the MRI sub-study will be asked at their 1-month follow-up visit about the occurrence of Adverse Events (AEs) related to the administration of MRI contrast (Gadolinium). These will be followed until resolution.

## Dissemination

The results of the study will be presented at national and international scientific meetings and published in full in a peer-reviewed journal (authorship will be according to that journal's guidelines). A lay summary will be produced and disseminated to interested parties.

## Trial Management

The trial will be coordinated from CRUK Glasgow CTU by the Trial Management Group (TMG), including the Chief Investigator, selected co-investigators, project manager, trial statistician, clinical trial co-ordinator and IT staff. The TMG will oversee the running of the trial and meet monthly.

## Acknowledgements

The authors are grateful to the Chief Scientist Office (Scotland) as the study funder, cancer research networks infrastructure, the participating sites and staff, SomaLogic Inc and the patients involved. ST is part funded by the West of Scotland Lung Cancer Research Fund. KGB is part funded by the NHS

Career Research Fellowship. CR-UK and ECMC are acknowledged by FT and CMcC.

## REFERENCES


1 Musk AW, Olsen N, Alfonso H, *et al.* Predicting survival in malignant mesothelioma. *Eur Respir J* 2011;**38**:1420–4. doi:10.1183/09031936.00000811

2 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung Cancer* 2015;**88**:344–8. doi:10.1016/j.lungcan.2015.03.005

3 Tsim S, Dick C, Roberts F, *et al.* 76 Early experience of a regional mesothelioma MDT in the West of Scotland. *Lung Cancer* 2014;**Supplement 1**:S28–9. doi:10.1016/S0169-5002(14)70076-5

4 The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. 1997;**111**:106–9.http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8996002&retmode=ref&cmd=prlinks

5 Scherpereel A, Astoul P, Baas P, *et al.* Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. Eur. Respir. J. 2010;**35**:479–95. doi:10.1183/09031936.00063109

6 Rusch VW, Giroux D. Do we need a revised staging system for malignant pleural mesothelioma? Analysis of the IASLC database. *Ann Cardiothorac Surg* 2012;**1**:438–48. doi:10.3978/j.issn.2225-319X.2012.11.10

7 Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: Diagnosis. 1993;**72**:389–93.http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8319170&retmode=ref&cmd=prlinks

8 Efficacy of pleural needle biopsy and pleural fluid cytopathology in the diagnosis of malignant neoplasm involving the pleura. 1975;**67**:536–9.http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=1126189&retmode=ref&cmd=prlinks

9 Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. 1985;**60**:158–64.http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=3974296&retmode=ref&cmd=prlinks

10 Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid examination. 1991;**4**:320–4.http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=2068057&retmode=ref&cmd=prlinks

11 A randomised controlled trial of intervention site radiotherapy in malignant pleural mesothelioma. 2007;**84**:18–22. doi:10.1016/j.radonc.2007.05.022

12 Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. 1995;**108**:754–8.http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7656629&retmode=ref&cmd=prlinks

13 Robinson BW, Creaney J, Lake R, *et al.* Mesothelin-family proteins and diagnosis of mesothelioma. *The Lancet* 2003;**362**:1612–6. doi:10.1016/S0140-6736(03)14794-0

14 Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax* 2014;**69**:895–902. doi:10.1136/thoraxjnl-2014-205205

15 Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. 2006;**12**:4225–31. doi:10.1158/1078-0432.CCR-06-0472

16 Creaney J, Yeoman D, Demelker Y, *et al.* Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. *J Thorac Oncol* 2008;**3**:851–7. doi:10.1097/JTO.0b013e318180477b

17 Pass HI, Lott D, Lonardo F, *et al.* Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med* 2005;**353**:1564–73. doi:10.1056/NEJMoa051185

18 Chang K, Pai LH, Batra JK, *et al.* Characterization of the antigen (CAK1) recognized by monoclonal antibody K1 present on ovarian cancers and normal mesothelium. *Cancer Res* 1992;**52**:181–6.

19 Chang K, Pai LH, Pass H, *et al.* Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg Pathol* 1992;**16**:259–68.

20 Hollevoet K, Nackaerts K, Thimpont J, *et al.* Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. *Am J Respir Crit Care Med* 2010;**181**:620–5. doi:10.1164/rccm.200907-1020OC

21 Hooper C, Lee YCG, Maskell N, *et al.* Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax. 2010;**65 Suppl 2**:ii4–17. doi:10.1136/thx.2010.136978

22 THe effect of clinical covariates on the diagnostic and prognostic value of soluble mesothelin and megakaryocyte potentiating factor. 2012;**141**:477–84. doi:10.1378/chest.11-0129

23 Inflammation: an important parameter in the search of prostate cancer biomarkers. 2014;**12**:32. doi:10.1186/1477-5956-12-32

24 Effects of rectal examination, prostatic massage, ultrasonography and needle biopsy on serum prostate specific antigen levels. 1992;**147**:810–4.

25 The effects of timing of fine needle aspiration biopsies on gene expression profiles in breast cancers. 2008;**8**:277. doi:10.1186/1471-2407-8-277

26 Pass HI, Levin SM, Harbut MR, *et al.* Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *N Engl J Med* 2012;**367**:1417–27. doi:10.1056/NEJMoa1115050

27 Kirschner MB, Pulford E, Hoda MA, *et al.* Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. *Br*

*J Cancer* 2015;**113**:963–9. doi:10.1038/bjc.2015.286

28 Ostroff RM, Mehan MR, Stewart A, *et al.* Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. *PLoS ONE* 2012;**7**:e46091. doi:10.1371/journal.pone.0046091

29 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004. doi:10.1371/journal.pone.0015004

30 Kao SCH, Pavlakis N, Harvie R, *et al.* High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. *Clin Cancer Res* 2010;**16**:5805–13. doi:10.1158/1078-0432.CCR-10-2245

31 Hooper CE, Lyburn ID, Searle J, *et al.* The South West Area Mesothelioma and Pemetrexed trial: a multicentre prospective observational study evaluating novel markers of chemotherapy response and prognostication. *Br J Cancer* 2015;**112**:1175–82. doi:10.1038/bjc.2015.62

32 Pinato DJ, Mauri FA, Ramakrishnan R, *et al.* Inflammation-Based Prognostic Indices in Malignant Pleural Mesothelioma. *Journal of Thoracic Oncology* 2012;**7**:587–94. doi:10.1097/JTO.0b013e31823f45c1

33 Circulating and tumor-infiltrating myeloid cells predict survival in human pleural mesothelioma. 2011;**117**:5234–44. doi:10.1002/cncr.26143

34 Zhang Y, Marmorstein LY. Focus on molecules: Fibulin-3 (EFEMP1). *Experimental Eye Research* 2010;**90**:374–5. doi:10.1016/j.exer.2009.09.018

35 Agha MA, El-Habashy MM, El-Shazly RA. Role of fibulin-3 in the diagnosis of malignant mesothelioma. *Egyptian Journal of Chest Diseases and Tuberculosis* 2014;**63**:99–105. doi:10.1016/j.ejcdt.2013.10.004

36 Corradi M, Goldoni M, Alinovi R, *et al.* YKL-40 and mesothelin in the blood of patients with malignant mesothelioma, lung cancer and asbestosis. *Anticancer Res* 2013;**33**:5517–24.

37 Creaney J, Francis RJ, Dick IM, *et al.* Serum Soluble Mesothelin Concentrations in Malignant Pleural Mesothelioma: Relationship to Tumor Volume, Clinical Stage and Changes in Tumor Burden. *Clin Cancer Res* 2011;**17**:1181–9. doi:10.1158/1078-0432.CCR-10-1929

38 Pairon J-C, Laurent F, Rinaldo M, *et al.* Pleural plaques and the risk of pleural mesothelioma. *J Natl Cancer Inst* 2013;**105**:293–301. doi:10.1093/jnci/djs513

39 Paris C, Thierry S, Brochard P, *et al.* Pleural plaques and asbestosis: dose- and time-response relationships based on HRCT data. *Eur Respir J* 2009;**34**:72–9. doi:10.1183/09031936.00094008

40 The British Thoracic Society guidelines on the investigation and management of pulmonary nodules. 2015;**70**:794–8. doi:10.1136/thoraxjnl-2015-207221

41 *Guidance for industry, bioanalytical methods validation*. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm 2013. http://ci.nii.ac.jp/naid/10016982382/

42 Frauenfelder T, Tutic M, Weder W, *et al.* Volumetry: an alternative to assess therapy response for malignant pleural mesothelioma? *Eur Respir J* 2011;**38**:162–8. doi:10.1183/09031936.00146110

## CONTRIBUTIONS

Selina Tsim

- Contribution to the conception or design of the work; data acquisition, analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Caroline Kelly

- Contribution to the design of the work; analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Laura Alexander

- Contribution to the design of the work and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that

questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Carol McCormick

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Fiona Thomson

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Rosie Woodward

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content

- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

John E Foster

- Contribution to the conception and design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

David B Stobo

- Contribution to the design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Jim Paul

- Contribution to the conception and design of the work; data analysis

or interpretation of data for the work

- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Nick A Maskell

- Contribution to the design of the work; data analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Anthony Chalmers

- Contribution to the conception and design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kevin G Blyth

- Principal contribution to the conception and design of the work; data acquisition, analysis and interpretation of data for the work
- Drafting the work
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

**FUNDING**

This work was supported by the Chief Scientist's Office of the Scottish Government (Project Grant ETM/285) and the West of Scotland Lung Cancer Research Group (Award September 2015). KGB is part-funded by NHS Research Scotland.

**COMPETING INTERESTS STATEMENT**

SomaLogic Inc. have provided funding for all SOMAscan assays.

## FIGURE LEGENDS

### Figure 1

Summary of the design of the DIAPHRAGM study. Figure 1(a) is intended to describe the optimal diagnostic pathway for the majority of patients who present with significant Pleural Effusion +/- pleural thickening or a pleural mass. Figure 1(b) describes the optimal diagnostic pathway for the minority of patients who present with an isolated Pleural Mass, but no significant fluid component. The pathway chosen is ultimately at the discretion of the investigating physician.

## TABLES

**Table 1. Outcome Measures used in the DIAPHRAGM study**

| Research Objective | Outcome Measures |
|---|---|
| **Primary**<br>To determine whether SOMAscan results and/or Fibulin-3 levels in blood at presentation can differentiate MPM from asbestos-exposed controls and patients with other causes of pleural effusion with a sufficient degree of sensitivity and specificity to be of routine clinical value | Serum SOMAscan<br>Plasma Fibulin-3<br>Final diagnosis reached |
| **Secondary**<br>To determine whether:<br>1. SOMAscan results and/or Fibulin-3 levels at presentation provide clinically useful prognostic information in MPM patients | Serum SOMAscan & plasma Fibulin-3 at presentation<br>Survival (from registration) |
| 2. early changes in SOMAscan and/or Fibulin-3 levels after diagnosis (at 3 months) are associated with a poorer prognosis in MPM | Serum SOMAscan & plasma Fibulin-3 3 months post-Dx<br>Survival (from registration) |
| **Exploratory**<br>To determine whether:<br>1. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour volume, defined by MRI | Serum SOMAscan<br>Plasma Fibulin-3<br>MPM tumour volume at MRI, defined using Myrian intrasense™ software |
| 2. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour angiogenesis (as defined by perfusion-based MRI biomarkers) | Serum SOMAscan<br>Plasma Fibulin-3<br>The following MRI biomarkers:<br>• MRI-ECE<br>• Redistribution rate contstant ($K_{ep}$)<br>• Elimination rate constant ($K_{el}$) |
| 3. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and pleural fluid at presentation in patients with MPM | SOMAscan and Fibulin-3 at presentation and at 1 month post-biopsy +/- drainage and pleurodesis |

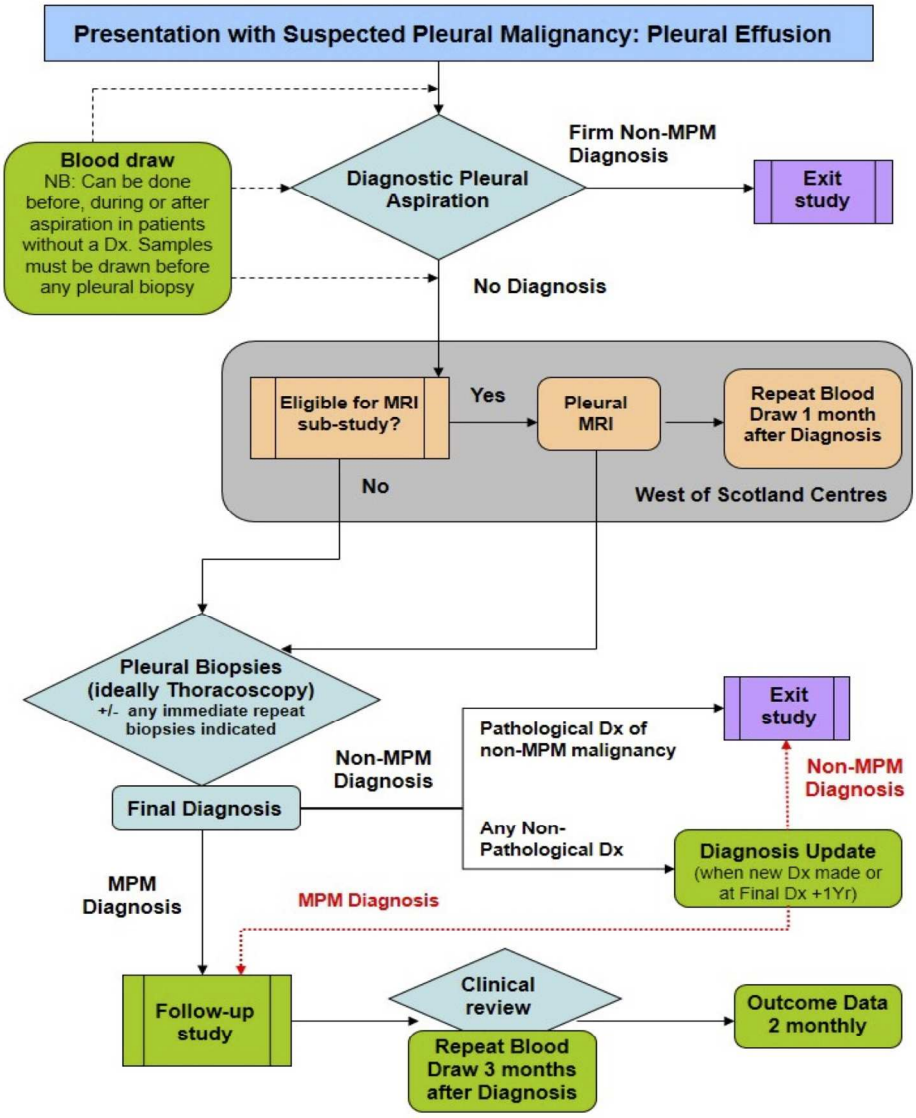



Figure 1(a)

156x180mm (300 x 300 DPI)

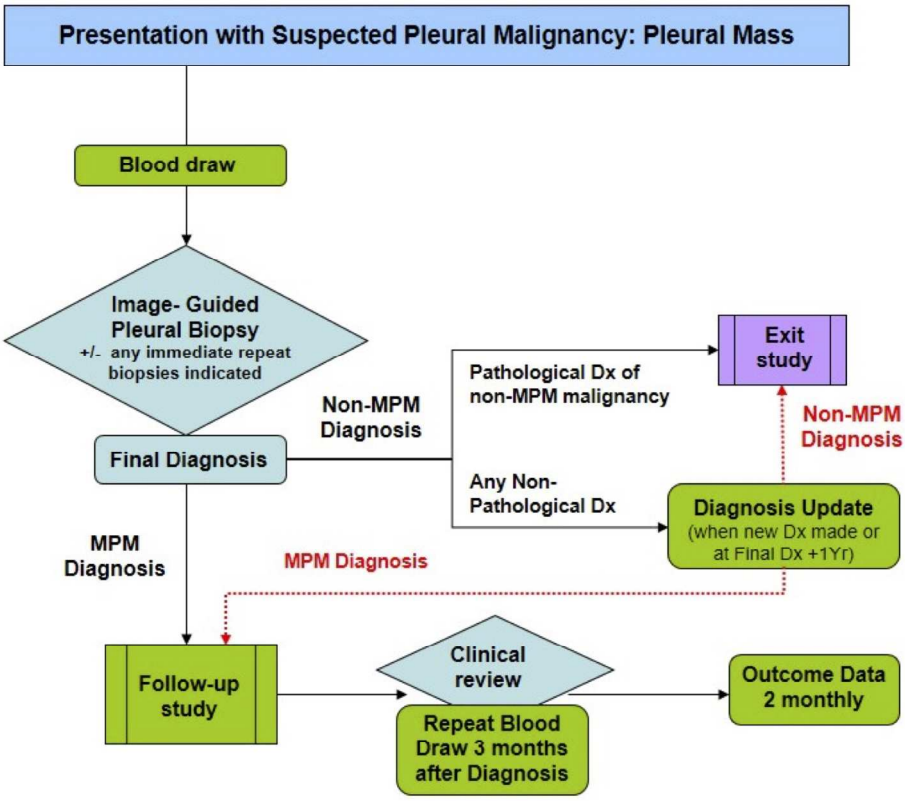



Figure 1(b)

166x144mm (300 x 300 DPI)

CANCER RESEARCH UK
Clinical Trials Unit, Glasgow

# ASBESTOS EXPOSURE QUESTIONNAIRE

(to be completed for all patients on study)

## DIAPHRAGM: Diagnostic and Prognostic biomarkers in the Rational Assessment of Mesothelioma

| | |
|---|---|
| **PATIENT INITIALS:** (f) _____ (s) _____ | **DATE of BIRTH:** DD / MON / YYYY |
| **INVESTIGATOR:** | **REGISTRATION DATE:** DD / MON / YYYY |
| **SITE:** | **PATIENT TRIAL IDENTIFIER:** |
| **DATE OF COMPLETION OF QUESTIONNAIRE:** DD / MON / YYYY | |

## Employment History — No known asbestos exposure ☐

| Industry / Occupation e.g. joiner, shipyard worker | Period of Employment: Start Year | No. of Years | No. of Months | No. of Hours per day | Days per week | Airway protection? | Job Type Please refer to codes below | Job Code Please refer to Page 2 | Indirect Exposure Details e.g. via husband, via father |
|---|---|---|---|---|---|---|---|---|---|
| **1.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **2.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **3.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **4.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **5.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |

**JOB TYPE:** **M** = Manufacturing asbestos products **S** = Asbestos Stripping/Removal **O** = Something else **I** = Indirect exposure

**DATE:** DD / MON / YYYY **INVESTIGATOR'S SIGNATURE**: _____

# Job Codes
## (Taken from Appendix 5 of the HSE Asbestos Survey)

### CODE LIST M

**Textile manufacture**
01 Raw material store
02 Raw material & finished product transport
03 Disintegrating/heating/opening/fibrising
04 Hopper feeding
05 Carding
06 Weaving
07 Spinning
08 Doubling/twisting
09 Braiding
10 Warping
11 Detritus handling
12 Inspecting
13 Supervising
14 Finished product store & dispatch
15 Other exposed workers
95 'Fortex' process

**Asbestos cement mixture board and pipe manufacture**
16 Supervising
17 Raw material store
18 Raw material transport
19 Disintegrating
20 Mixing/beating
21 Wet board or pipe manufacture
22 Wet board or pipe handling
23 Drying
24 Dry board handling
25 Machining or cutting
26 Sanding
27 Inspecting
28 Finished product store/packing/dispatch/transport
29 Detritus handling wet
30 Detritus handling dry
31 Other exposed worker

**Manufacture of asbestos/rubber/resin bitumen mixtures**
32 Raw material store
33 Transporting raw materials
34 Disintegrating
35 Handling raw fibre (bag tipping/weighting/mixing)
36 Pressing/moulding
37 Cutting/finishing/machining
38 Transporting finished product
39 Finished product storage/packing/dispatching
40 Inspecting
41 Supervising
42 Other exposed worker

**Asbestos board and paper manufacture**
43 Raw material store
44 Raw material transport
45 Disintegrating/opening/fibrising
46 Mixing/beating
47 Handling wet mixture
48 Drying
49 Handling dry mixture
50 Cutting/machining
51 Store/transport/packing/dispatching products
52 Supervising
53 Detritus handling
54 Inspecting
55 Other exposed workers

**Garment manufacture**
56 Cloth store
57 Cutting out
58 Stitching
59 Transport of materials
60 Storing/packing/dispatching
61 Inspecting
62 Supervising
63 Other exposed workers

**Manufacture of dry mixes for insulation & plastering**
70 Raw material stores
71 Raw material handling/bag tipping/weighing/mixing
72 Packaging
73 Stores and dispatch
74 Other exposed workers

**Maintenance workers all manufacturing sectors**
75 Supervising
76 Fault finder/machine fitter/installation engineer/plant engineer
77 Labour to plant engineers etc
78 Carpenters/joiners
79 Electrician
80 Plumber
81 Other building trade craftsmen eg painter
82 Labourer to building trade craftsman
83 Ventilation plant servicing
84 Factory cleaning

### CODE LIST S

**Asbestos stripping/removal**
64 Supervising
65 Stripping/encapsulating
69 Other exposed workers (eg sampler, cleaner, scaffolder)

### CODE LIST O

**Ship building, repair, & breaking**
85 Asbestos storeman
86 Lagging
87 Boilermakers and installers
88 Carpenters/joiners
89 Plumber
90 Engine fitter
91 Other exposed workers
92 Asbestos stripping
93 Cleaner
94 Shipbreaking

**Building & construction**
96 Heating engineer
97 Asbestos board cutting/fitting
98 Asbestos roofing construction and maintenance
99 Demolition
104 Other building trade craftsmen eg painter *(81)*†
105 Labourer to building trade craftsman *(82)*
106 Plumber *(80)*
107 Carpenters/joiners *(78)*

**Miscellaneous processes**
101 Use of asbestos string/rope/felt *(65)*
102 Fitting clutch and brake pads *(37)*
108 Machining/cutting asbestos/resin board *(50)*
109 Other exposed workers *(69)*

BMJ Open

# Diagnostic and prognostic biomarkers in the rational assessment of mesothelioma (DIAPHRAGM) study: protocol of a prospective, multi-centre, observational study

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2016-013324.R2 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 24-Oct-2016 |
| Complete List of Authors: | Tsim, Selina; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Cancer Sciences<br>Kelly, Caroline; Cancer Research UK Glasgow Clinical Trials Unit<br>Alexander, Laura; Cancer Research UK Glasgow Clinical Trials Unit<br>McCormick, Carol; Cancer Research UK Glasgow Clinical Trials Unit<br>Thomson, Fiona; Cancer Research UK Glasgow Clinical Trials Unit<br>Woodward, Rosemary; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility<br>Foster, John; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility<br>Stobo, David; Queen Elizabeth University Hospital, Radiology<br>Paul, Jim; Cancer Research UK Glasgow Clinical Trials Unit<br>Maskell, Nick; University of Bristol, Academic Respiratory Unit, School of Clinical Sciences; North Bristol NHS Trust, Respiratory Research Unit<br>Chalmers, Anthony; University of Glasgow, Institute of Cancer Sciences; Beatson West of Scotland Cancer Centre<br>Blyth, Kevin; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Infection, Immunity and Inflammation |
| Primary Subject Heading: | Respiratory medicine |
| Secondary Subject Heading: | Oncology, Patient-centred medicine, Research methods |
| Keywords: | Mesothelioma, Biomarker, Diagnosis, Prognosis |
| | |

**TITLE:**

# DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN THE RATIONAL ASSESSMENT OF MESOTHELIOMA (DIAPHRAGM) STUDY: PROTOCOL OF A PROSPECTIVE, MULTI-CENTRE, OBSERVATIONAL STUDY

**AUTHORS:**

Selina Tsim[1/2], Caroline Kelly[3], Laura Alexander[3], Carol McCormick[3], Fiona Thomson[3], Rosie Woodward[4], John E Foster[4], David B Stobo[5], Jim Paul[3], Nick A Maskell[6], Anthony Chalmers[2/7], Kevin G Blyth[1/8]

**Affiliations**

1. Department of Respiratory Medicine, Queen Elizabeth University Hospital, Glasgow, U.K.
2. Institute of Cancer Sciences, University of Glasgow, U.K.
3. Cancer Research UK Glasgow Clinical Trials Unit, UK
4. Glasgow Clinical Research Imaging Facility, Queen Elizabeth University Hospital, Glasgow, U.K.
5. Department of Radiology, Queen Elizabeth University Hospital, Glasgow, U.K.
6. Academic Respiratory Unit, School of Clinical Sciences, University of Bristol, UK
7. Beatson West of Scotland Cancer Centre, Glasgow, UK
8. Institute of Infection, Immunology and Inflammation, University of Glasgow, U.K.

**Corresponding Author and Address**

Kevin G. Blyth

Consultant Respiratory Physician and NRS Career Research Fellow

Department of Respiratory Medicine, Administration Building,

Queen Elizabeth University Hospital,

Glasgow, G51 4TF

kevin.blyth@ggc.scot.nhs.uk

Tel: +44 141 451 6099

**Keywords:**

Mesothelioma

Biomarker

Diagnosis

Prognosis

**WORD COUNT:**

3998/4000

## ABSTRACT (300/300)

### INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer, which is difficult to diagnose. Thoracoscopy is frequently required but is not widely available. An accurate, non-invasive diagnostic biomarker would allow early specialist referral, limit diagnostic delays and maximize clinical trial access. Current markers offer insufficient sensitivity and are not routinely used. The SOMAmer® proteomic classifier and Fibulin-3 have recently demonstrated sensitivity and specificity exceeding 90% in retrospective studies. DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma) is a suitably powered, multi-centre, prospective observational study designed to determine whether these markers provide clinically useful diagnostic and prognostic information.

### METHODS AND ANALYSIS

Serum and plasma (for SOMAscan® and Fibulin-3, respectively) will be collected at presentation, prior to pleural biopsy/pleurodesis, from 83-120 MPM patients, 634-724 patients with non-MPM pleural disease and 109 asbestos-exposed controls. Final numbers of MPM/non-MPM cases will depend on the incidence of MPM in the study population (estimated at 13-20%). Identical sampling and storage protocols will be used in 22 recruiting centres and histological confirmation sought in all cases. Markers will be measured using the SOMAscan proteomic assay (SomaLogic Inc.) and a commercially available Fibulin-3 ELISA (USCN Life Science Inc.). The standard error in the estimated sensitivity and specificity will be <5% for each

marker and their performance will be compared to serum Mesothelin. Blood levels will be compared to paired pleural fluid levels and MPM tumour volume (using Magnetic Resonance Imaging) in a nested sub-study. The prognostic value of each marker will be assessed and a large bioresource created.

## ETHICS AND DISSEMINATION

The study has been approved by the West of Scotland Research Ethics Committee (Ref: 13/WS/0240). A Trial Management Group meets on a monthly basis. Results will be published in peer-reviewed journals, presented at international meetings and disseminated to patient groups.

**TRIAL REGISTRATION NUMBER:** ISRCTN10079972

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- Prospective, multi-centre study recruiting a representative sample of patients in an intention-to-diagnose population
- Strict sampling, processing and storage methods used in all patients
- Robust diagnostics and 12 months' follow-up
- Creation of a large bio-resource annotated with detailed, prospectively collected clinical information, for use in future biomarker discovery and validation studies
- The final number of study participants with MPM, and therefore the power available to test the primary objective, will not be known until recruitment is complete.

## INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an invasive thoracic malignancy, strongly associated with prior asbestos exposure. The median survival for patients with MPM is poor at 9-10 months [1,2]. However, the prognosis of individuals is highly variable and largely determined by histological subtype [2]. MPM frequently presents as an emergency with a large, symptomatic pleural effusion [3]. Early specialist referral is frequently required because pleural fluid aspiration cytology is unreliable [4] and histological confirmation is recommended in all patients [5]. Thoracoscopy (under local or general anaesthesia) [5], enables widespread tissue sampling [6] with diagnostic yields for malignancy >90% [7] but is not available in all centres. Thoracoscopy also allows pleurodesis or indwelling pleural catheter placement.

A reliable, non-invasive diagnostic biomarker for MPM would be a major clinical advance. This would allow clinicians to reliably differentiate likely MPM from secondary pleural malignancies (e.g. lung or breast cancer), which may present with similar clinical and imaging features but require less evolved diagnostic pathways. This reflects the improved sensitivity of pleural cytology in these diseases [8-10] and the frequent option of alternative sites for tissue biopsy. A positive MPM biomarker test could facilitate early referral to a thoracoscopy centre and avoid unnecessary diagnostic delay (e.g. due to repeated pleural aspirations), minimising the risk of subsequent needle-tract metastases [11,12] and maximizing opportunity for clinical trial enrolment. Previous studies have demonstrated that blood levels of single proteins,

including mesothelin [13,14], megakaryocyte potentiating factor (MPF) [15] and osteopontin [16], are higher in patients with MPM than in asbestos-exposed controls (AECs) and patients with secondary pleural malignancies. Mesothelin, a cell-adhesion glycoprotein that is over-expressed in MPM [17,18] is the most widely studied and is associated with an MPM sensitivity of 56-77% at 95% specificity [14,15,19] but much reduced performance in patients with non-epithelioid MPM. A recent meta-analysis (of 4491 individuals (1026 with MPM)) reported a sensitivity of only 32% at 95% specificity. Mesothelin does not, therefore, contribute to current diagnostic algorithms [20]. MPF offers no advantage over mesothelin [15], while the clinical utility of osteopontin is limited by stability and reproducibility concerns [16].

An ideal MPM biomarker would be measurable in blood for ease of collection and offer sufficient sensitivity at high specificity in patients presenting with suspected MPM. Differentiation between advanced disease patients and appropriate controls is of limited value. High specificity is mandatory for a low prevalence disease, and should apply to patients with asbestos exposure and non-MPM pleural disease. Biomarker results should also correlate with disease extent and have defined relationships with potential confounders including renal function [21] and the effect of pleural interventions. The latter is important because the precedent has been established in prostate [22,23] and breast cancer [24], that recent sampling, resection or peri-tumoural inflammation may affect biomarker expression. This is particularly relevant to MPM where biopsies are frequently large and often combined with pleurodesis. Several previous biomarker studies, which validated

inconsistently in external populations, used samples acquired at later time-points, often post-diagnosis (and post-pleurodesis) including samples taken prior to, during, or after resection surgery [16,25,26]. The aim of the DIAPHRAGM study is to prospectively evaluate the diagnostic and prognostic performance of the SOMAscan proteomic classifier [27] and fibulin-3 [25], which have demonstrated high sensitivity and specificity in recent retrospective series. The study has been designed to generate clinically meaningful results, which can be related to MPM biology and confounding factors, and applied to patients at first presentation.

**SOMAmer-based Proteomic Classifier**

The SOMAscan assay is a highly multiplexed proteomic platform that utilizes SOMAmer (Slow Off-rate Modified Aptamers) reagents to selectively bind and quantify proteins [28]. A 13-protein classifier was developed by SomaLogic Inc. (Boulder, Colorado), using this novel proteomics-based biomarker detection technique [27] in a retrospective study over 800 proteins were measured in the serum of 117 MPM patients and 142 AECs, collected at surgical MPM centres in the US between 1996 and 2011. Using a panel of 13 differentially expressed proteins and a cut-point of 0.5, the classifier was able to segregate MPM from controls with an area under the curve (AUC) of 0.99 +/- 0.01 in training (60 MPM/60 controls), 0.98 +/- 0.04 in blinded verification (19 MPM/20 controls) and 0.95 +/- 0.04 in blinded validation sets (38 cases/62 controls) [27]. The combined sensitivity for the three cohorts was 93% at 91% specificity. Based on the published ROC curve for the validation cohort, sensitivity at 95% specificity appeared to be approximately 78%,

although the authors did not report this value. This performance exceeds that of any previous MPM biomarker, although the classifier's specificity appeared lower in patients with non-MPM pleural effusion (n=32). There was a modest correlation between classifier score and disease stage, but prognostic significance was not assessed. The 13 classifier proteins (nine up-regulated, four down-regulated) have not previously been associated with MPM. Their functions fall into two broad groups; regulation of proliferation and inflammation. Quite apart from their biological relevance to MPM, the latter is an important potential confounder because many of the patients involved will have previously undergone pleurodesis. In addition, several groups have reported an independent interaction between prognosis and inflammatory biomarkers in MPM, including neutrophil-to-lymphocyte ratio (NLR) [29-31], monocytosis [32] and the modified Glasgow Prognostic Score [31]. Therefore, adequate understanding of the diagnostic and prognostic utility of this assay requires replication in a pre-pleurodesis cohort and prospective evaluation of interactions between inflammatory biomarkers and SOMAscan scores.

**Fibulin-3**

Fibulin-3 is a secreted glycoprotein, encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [33]. Fibulin-3 is over-expressed in MPM tumours relative to adjacent benign pleura [25] and expressed and secreted by MPM cell lines [26]. Pass et al retrospectively measured fibulin-3 in the plasma of 92 MPM patients, 136 AECs, 93 patients with non-MPM pleural effusion and 43 healthy controls [25].

A plasma cut-point of 52 ng/ml provided 97% sensitivity at 95% specificity and a 95% CI of the AUC of 0.97-0.99 in differentiating MPM from all other cases. However, in a blinded external validation set, sensitivity was below 40% (at 95% specificity), with an AUC=0.87.

Subsequent studies have revealed mixed results. In a study of 153 patients (82 with MPM), Creaney et al reported a sensitivity of 22% (at 95% specificity) at the same 52 ng/ml cut-point and an AUC of 0.671 (0.606 to 0.732), which was significantly inferior to mesothelin measured in the same patients (sensitivity 56% (at 95% specificity); AUC 0.816 (0.755 to 0.867)) at a 2.5 nM threshold [14]). In a small Egyptian study using an unspecified Fibulin-3 assay and internally-defined cut-points, Agha et al reported 100% sensitivity/78% specificity in differentiating MPM cases (n=25) from non-malignant pleural disease (n=9), and 88% sensitivity/82% specificity in differentiating MPM from secondary pleural malignancies (n=11) [34]. No combined sensitivity was reported. An Italian study found no difference in Fibulin-3 levels but used serum (not plasma), a control group without pleural disease (Asbestosis) and contained only 14 patients with MPM [35].

## METHODS AND ANALYSIS

### Study Design

DIAPHRAGM is a prospective, multi-centre observational study. The study incorporates sampling windows that correspond to the proposed use of a diagnostic biomarker, i.e. at presentation with Suspected Pleural Malignancy (SPM). The overall study design is summarized in Figure 1(a) and 1(b). The

main impact of this design is that biomarkers will be drawn before a diagnosis is made. In addition to better replicating the future use of these markers, this avoids the potential confounding effect of pleurodesis on biomarker results. The diagnostic performance of the SOMAmer panel and Fibulin-3 will be assessed using cut-points determined in the relevant original studies and compared to mesothelin (using the MESOMARK® ELISA (Fujirebio Diagnostics Inc, PA, USA). Identical processing and storage protocols will be used in patients with SPM and a group of AECs. Potential confounders including renal function, inflammatory indices and drugs will be recorded at all visits. The timing of the biomarker blood draw in relation to pleural aspiration (pre-aspiration or post-aspiration) will be recorded in order to assess the effect of this intervention on biomarker results. An exploratory, cross-sectional Magnetic Resonance Imaging (MRI) sub-study will determine if there is any correlation between blood biomarker levels and MPM tumour volume, as has been established for Mesothelin using Computed Tomography-Positron Emission Tomography scanning [36].

**Study Objectives and Outcome Measures**

These are presented in Table 1.

**Setting**

At least 600 consecutive patients with SPM will be recruited from 21 centres (20 in the UK, 1 in Republic of Ireland). These are a mixture of academic and more clinically orientated units. This should make the results of the DIAPHRAGM study generalizable to patients presenting with SPM to acute

hospital services. The principal criterion used to select centres was that they had sufficiently evolved pleural diagnostic services to deliver a reliable diagnosis. Specifically, access to on-site thoracoscopy (ideally including local anaesthetic thoracoscopy (LAT)) and a regional mesothelioma MDT meeting (for diagnostic review and staging) was required.

**Screening and Eligibility Assessment**

Suspected Pleural Malignancy

Cases will be identified on presentation to a Respiratory out-patient clinic or acute hospital admissions unit. This will be based on the history, examination and available investigations. Potentially eligible patients will be provided with the study Patient Information Sheet (PIS, see Online Supplementary Appendix 1) and eligibility assessed based on the following criteria:

*Inclusion Criteria:*

- SPM, defined by a unilateral pleural effusion or pleural mass lesion
- Sufficient fitness for diagnostic sampling (site investigator's clinical judgment)
- Informed written consent

*Exclusion Criteria:*

- Intercostal chest drain in-situ, or inserted within the previous 3 months

Asbestos-related pleural plaques are not an inclusion criterion since these are absent in up to 25% of MPM cases [37], and are also common in asbestos-

exposed populations without MPM [38]. Patients with lung nodules or other visceral mass lesions are not excluded, assuming the investigator suspects pleural malignancy. This is because of the high prevalence of lung nodules in the target population (older patients, commonly smokers) and the high false positive rate of CT imaging in this regard [39].

Subjects recruited to the SPM arm will generate cohorts of MPM and non-MPM pleural disease of various aetiologies, likely including Benign Asbestos-related Pleural Effusion and secondary pleural malignancies. These numbers will be sufficient to address the primary objective with sufficient statistical power to inform clinical practice (see later section).

Asbestos-exposed control (AEC) subjects

109 AECs will be recruited via invitations sent by Clydeside Action on Asbestos (CAA), an advocacy body based in Glasgow with a database of over 600 clients, or by Respiratory clinics at the host centre. Individuals will be invited to participate by letter (if identified via CAA) or given the PIS (see Online Supplementary Appendix 2) at clinic. All subjects will be invited to a single research clinic visit assuming the following Eligibility Criteria are met.

*Inclusion Criteria:*

- Documented history of asbestos exposure and associated pleural plaques, asbestosis or diffuse pleural thickening
- Willing and able to travel to a research clinic interview in Glasgow
- Informed written consent

*Exclusion Criteria:*

- Known MPM
- Known or suspected other thoracic malignancy under investigation
- Known pleural effusion of any cause

Detailed asbestos exposure histories will be taken from all participants in both the suspected pleural malignancy cohort and the asbestos-exposed control cohort. This will be done using an asbestos exposure questionnaire derived from Health and Safety Executive asbestos survey [40] (see Online Supplementary Appendix 3). This questionnaire includes recording of the nature of occupational exposure(s), which can be correlated to likely fibre exposure. The duration and first year of exposure is also recorded. Non-occupational sources of exposure are also recorded (e.g. the washing of an occupationally exposed spouse's work clothes). Only AECs with documented imaging sequelae of asbestos exposure (e.g. pleural plaques) and an asbestos exposure history will be included.

Cross-sectional MRI sub-study

50 patients will be recruited to address the study's exploratory objectives (see Table 1). Eligibility will be determined based on the following criteria.

*Inclusion Criteria:*

- Pleural histological sampling (by LAT/image-guided biopsy) indicated to investigate SPM following a non-diagnostic pleural aspiration
- Recruited in a West of Scotland centre

*Exclusion Criteria:*

- Unable to undergo MRI (claustrophobia or known contraindications such as pacemaker, ferrous metal implants or foreign body)
- Allergy to Gadolinium contrast
- Renal impairment (eGFR <30ml/min)
- Pregnancy

Based on previous audit data from the host centre we expect at least 40% (n=20) of patients in the sub-study to have MPM. Eligible subjects will be approached at the clinical visit during which non-diagnostic pleural aspiration results, and the need for further investigation, are discussed. Subjects will be provided with a separate PIS (see Online Supplementary Appendix 4) and will be asked to provide additional informed written consent.

**Consent**

All subjects will be given sufficient time (as judged by themselves) to provide written informed consent after reading the relevant PIS and having the opportunity to ask questions.

**Outcome Measures**

The outcome measures associated with each of the trial's objectives are detailed in Table 1.

*Final Diagnosis*

A specific cytological or histological pleural diagnosis will be sought in all patients according to national guidelines [20]. This will be recorded as the Final Diagnosis, which may be based on immediate repeat biopsies felt to be indicated by the site PI (see Figure 1). Any cytologically or histologically confirmed non-MPM diagnosis (e.g. pleural metastases from lung cancer) will be recorded without the need for any further updates. However, sites will need to provide updates for any non-MPM diagnosis that is not cytologically or histologically confirmed (e.g. parapneumonic effusion). These will be submitted on the 12-month anniversary of the original diagnosis, or as soon as any new pleural diagnosis is made. This aims to capture any false negative diagnostic tests from the initial presentation, acknowledging the major diagnostic challenges posed by pleural malignancies, particularly MPM.

*Biomarker Sampling and Storage*

Blood samples (+/- pleural fluid in WoS centres) will be drawn and immediate processing performed at each study centre. Samples can be taken before or after pleural aspiration. Patients with positive pleural cytology cannot be recruited (see Figure 1(a)). Duplicate samples will be collected for all measurements at all visits, ensuring redundancy in case of loss or damage to samples during transportation to the appropriate central laboratory. SOMAmer biomarker levels will be measured in serum; therefore, 9 ml of venous blood will be collected first into a vacutainer tube containing SST clot activator. Fibulin-3 levels will be measured in plasma; therefore, 9 ml of venous blood will be collected second into a vacutainer tube containing EDTA. In centres contributing to the exploratory MRI sub-study (WoS sties

only) 20 ml of pleural fluid will be also collected into a plain container if pleural fluid is being drawn for diagnostic/therapeutic purposes at the same visit. If not done at this first opportunity, pre-diagnosis pleural fluid can also be collected during local anaesthetic or general anaesthetic thoracoscopy, prior to any biopsy or pleurodesis being performed.

*Biomarker Processing and Storage*

Serum samples will be allowed to clot for 30 minutes before centrifugation. Plasma and pleural fluid samples will be centrifuged immediately. All samples will be centrifuged at 2200g for 15 minutes at room temperature. For all samples, the supernatant will be withdrawn by pipette, aliquoted into cryovials of at least 250μL volume, labeled and placed into a -80 freezer within 2 hours. Samples will be stored at each recruiting centre until batched transport to the appropriate study laboratory. Samples from WoS recruiting centres will be used to create a bioresource. The bioresource will be stored as a satellite collection of the NHS Greater Glasgow & Clyde Biorepository, a Health Improvement Scotland (HIS)-approved tissue bank. Data will be stored in the secure Cancer Research UK Clinical Trials Unit database. On study completion, investigators will be invited to apply for access to data and samples appropriate to their research questions. This will allow external validation of new markers, including those reported since the study's design (such as High Mobility Group Box-1 (HMGB-1)) [41], in an intention to diagnose population, Access will be granted after peer review of each proposal by a scientific board comprising members of the DIAPHRAGM TMG

and senior Biorepository staff. An annual update on this activity will be submitted to the West of Scotland Research Ethics Committee.

*Biomarker Analyses*

SomaLogic Inc. (Boulder, Colorado, USA) will perform all SOMAscan proteomic analyses [27]. This utlises SOMAmer reagents to specifically bind to protein targets in blood. Relative protein concentrations will be converted to measurable nucleic acid signals that are quantified by hybridization to DNA microarrays [28].

Fibulin-3 and mesothelin levels will be measured by the Translational Pharmacology Unit, Wolfson Wohl Cancer Research Centre, UK, using ELISA methods validated according to the FDA-recommended guidelines for bioanalytical methods [42]. Fibulin-3 levels in plasma and pleural fluid will be measured using the commercially available ELISA (Cloud-Clone Corp., formerly USCN Life Science Inc, Houston, Texas, USA) as in the original Pass study [25]. Mesothelin will be measured using the MESOMARK® ELISA (Fujirebio Diagnostics, Inc, PA, USA).

*Magnetic Resonance Imaging*

Patients will be scanned at the Queen Elizabeth University Hospital, Glasgow, on a 3.0T Siemens Verio MRI Scanner. After localisation of the affected thoracic cavity, an isotropic 3D T1-weighted volume will be acquired using VIBE sequences. A stack of axial slices covering the entire lung and surrounding pleura will be acquired as a set of short breath-holds. Gd-DTPA

contrast (Gadovist) will be administered via a peripheral intravenous line as a 15-40 ml bolus (0.05 mmol/kg). VIBE sequences will be reacquired at copied slice positions to provide pre and post-contrast images. The total scan time will be around 45 minutes. Regions of enhancing pleural tumour will be defined using semi-automated signal intensity thresholding based on contrast-enhanced axial slices using Myrian Intrasense™ software, which has previously been used to assess tumour volume in MPM. [43] MRI volumetry analyses will be validated using imaging phantoms.

*Survival*

Survival will be recorded in days from the date of study registration to the data of death, from any cause.

**Sample Size, Assumptions and Uncertainties**

Sample size estimations for each marker were based on published data at the point of study design and a projected MPM incidence of 13-20% in the SPM cohort. The power available to test the hypotheses below is therefore reported as a range, based on final MPM numbers lying between 83 (13% incidence) and 120 (20% incidence).

Primary Objective

*SOMAscan Assay*

We hypothesize that the MPM sensitivity and specificity exceed 90%, based on previously reported performance in combined training, verification and validation sets (sensitivity 93.2% (88.6–97.7%), specificity (90.8% (86.1–

95.6%) [27]). Recruitment of 83-120 MPM patients will allow us to distinguish a sensitivity of >90% from a sensitivity <80% with 80-93% power, respectively, at the 5% 1-sided level of significance. 83-120 MPM patients will allow discrimination between a specificity <80% and a specificity >90%, with 80-88% power at the 5% 1-sided level of statistical significance. The standard error in the estimated sensitivity and specificity will be less than 5%, across all possible outcomes.

*Fibulin-3*

We hypothesize that the MPM sensitivity will exceed 80% and that the specificity will exceed 90% (at the 52 ng/ml cutoff). These figures are based on a reduced level of performance to the primary results reported by Pass et al (97% sensitivity, 95% specificity), given lower sensitivity in the external validation cohort studied (40% at 95% specificity) [25].

With 83-120 MPM patients the study will be able to distinguish a sensitivity of >80% from a sensitivity <70% with 65-80% power, respectively, at the 5% 1-sided level of statistical significance. The standard error in the estimated sensitivity will be less than 5%. In order to achieve 90% power to distinguish a specificity of >90% from a specificity <85% at the 5% 1-sided level of statistical significance, a random sample of 378 non-MPM samples will be analysed. The standard error in the estimated specificity will be <2.3%.

The study data will be used to estimate the AUC for the SOMAscan marker for distinguishing MPM from non-MPM patients in the SPM cohort. Assuming

83-120 patients in the MPM group and 83-120 in the non-MPM group the AUC can be estimated with a 95% confidence interval of width 0.120-0.168 (assuming a cut-point exists with a reasonable sensitivity of 80% and a modest specificity of 40%). If more sensitive/specific cut-points exist the width of the 95% confidence interval will be much reduced. The study data will be used to develop a new diagnostic signature based on Fibulin-3 and SOMAscan results to distinguish MPM from non-MPM effusions.

Secondary Objectives

The study data will be used to determine whether baseline SOMAscan results and/or fibulin-3 levels, or a change in levels at 3 months (Fibulin-3 only), are independent prognostic factors for MPM. A correlation of 0.4 between existing prognostic factors and each marker has been assumed. For the baseline levels, to detect an approximate doubling in median OS (from 6 month to 12 months - a hazard ratio of 2) with 80% power and 5% 2-sided level of statistical significance between a good/poor prognostic group based on dichotomising these markers requires at least 83 MPM patients recruited over three years with approximately 6 months subsequent follow-up to observe 66 deaths. For the 3-month change levels, a hazard ratio of 2.38 can be detected (80% power, 5% 2-sided level of statistical significance) when 49 deaths are observed in the estimated 66 out of 83 patients who survive to 3 months.

Exploratory Objectives

These will be addressed in the MRI sub-study, which will generate a sample of at least 20 MPM patients. This will allow moderately large associations (0.6) between the exploratory outcome measures (see Table 1) to be detected at 80% power at the 5%, two-sided level of statistical significance. The effect of pleural biopsies +/- drainage/pleurodesis on Fibulin-3 levels will be assessed using all 50 patients recruited. This will allow moderately small differences (standardised difference of 0.4) to be detected with 80% power at the 5% two-sided level of statistical significance.

**Statistical Analysis Plan**

Primary Analysis

Sensitivity and specificity at pre-specified cut-offs will be estimated using standard approaches for proportions. The diagnostic performance of each biomarker will be assessed using ROC curves. All patients with MPM (n=83-120) will be included and compared with AECs and a random sample of non-MPM cases. Due to cost constraints related to SOMAscan analyses 83 AECs and 83 non-MPM cases will be randomly selected. All AECs and 378 non-MPM cases will be used for Fibullin-3 analyses and for comparison with Mesothelin. Logistic regression will be used to estimate a diagnostic model using biomarker results and clinical or radiological variables. Cross validation will be used to provide robust estimates of AUC and specificity at fixed sensitivity rates of 80%, 90% and 95%.

Secondary Analysis

A prognostic model will be developed using Cox proportional hazard techniques. The modelling process will incorporate biomarker measurements (at presentation (both markers) and at 3 months (Fibulin-3 only) and other known prognostic features (e.g. performance status, histology).

Exploratory Analysis

The association between SOMAscan results/fibulin-3 in blood and tumour volume/measures of tumour angiogenesis will be estimated by Pearson or Spearman correlation, depending on the normality of the data. The same methods will be used to test the association between fibulin-3 in blood and pleural fluid. Changes in Fibulin-3 levels before and after histological sampling (at 1 month follow-up) will be compared using a paired t-test or Wilcoxon signed rank sum test (depending on the normality of the data). Due to cost constraints, exploratory end-points involving pleural fluid SOMAscan results will be analysed at a later date.

**Changes to the Study Protocol since Trial Opening**

The protocol described accurately reflects Version 5, of the protocol, dated 17/6/16. The following changes were made in previous versions:

- Version 2, dated 14/2/14:
  - Safety reporting reduced following risk assessment by study Sponsor.
  - Collection of duplicate blood samples as provision for loss or damage and for sample retention in tissue bank.
  - Greater flexibility to timing of first blood draw.
- Version 3, dated 17/10/14:

- Addition of recruitment of Controls from Respiratory Medicine clinics
- Addition of exclusion criteria for patients with chest drains in-situ.
- Eligibility for the MRI sub-study extended to patients proceeding to image-guided pleural biopsy

- Version 4, dated 27/4/15:
  - Update to the exclusion criteria for the AECs to include known or suspected thoracic malignancy under investigation.
- Version 5, dated 17/6/16:
  - Power projections adjusted based on interim reporting of MPM incidence from recruiting centres.

**Definition of End of Study**

The trial will end 2 years after the last patient with confirmed MPM is recruited or whenever all patients with MPM have died (whichever occurs first).

## ETHICS AND DISSEMINATION

### Ethics

The study protocol, all documents and amendments have been approved by the West of Scotland Research Ethics Service (Ref: 13/WS/0240).

### Monitoring, Data Management and Quality Assurance

No on-site monitoring will be undertaken. Two telephone-monitoring calls will be conducted by a CRUK Glasgow CTU Monitor to carry out process, compliance and documentation checks. Central monitoring of trial data will be performed by the Trial Statistician and Clinical Trial Co-ordinator by checking

incoming forms for compliance with the protocol, data consistency, missing data and timing. The CRUK Glasgow CTU will control data consistency and data quality by entering trial data onto CTU database. Computerised and manual consistency checks will be performed and queries issued in cases of inconsistency or missing information. An audit trail of changes to the database will be maintained.

## Safety Considerations

Participants in the MRI sub-study will be asked at their 1-month follow-up visit about the occurrence of Adverse Events (AEs) related to the administration of MRI contrast (Gadolinium). These will be followed until resolution.

## Dissemination

The results of the study will be presented at national and international scientific meetings and published in full in a peer-reviewed journal (authorship will be according to that journal's guidelines). A lay summary will be produced and disseminated to interested parties.

## Trial Management

The trial will be coordinated from CRUK Glasgow CTU by the Trial Management Group (TMG), including the Chief Investigator, selected co-investigators, project manager, trial statistician, clinical trial co-ordinator and IT staff. The TMG will oversee the running of the trial and meet monthly.

## Acknowledgements

The authors are grateful to the Chief Scientist Office (Scotland) as the study funder, cancer research networks infrastructure, the participating sites and staff, SomaLogic Inc and the patients involved. ST is part funded by the West of Scotland Lung Cancer Research Fund. KGB is part funded by the NHS Career Research Fellowship. CR-UK and ECMC are acknowledged by FT and CMcC.

## REFERENCES


1 Musk AW, Olsen N, Alfonso H, *et al.* Predicting survival in malignant mesothelioma. *Eur Respir J* 2011;**38**:1420–4. doi:10.1183/09031936.00000811

2 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung Cancer* 2015;**88**:344–8. doi:10.1016/j.lungcan.2015.03.005

3 Tsim S, Dick C, Roberts F, *et al.* 76 Early experience of a regional mesothelioma MDT in the West of Scotland. *Lung Cancer* 2014;**Supplement 1**:S28–9. doi:10.1016/S0169-5002(14)70076-5

4 Renshaw AA, Dean BR, Antman KH, *et al.* The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 1997;**111**:106–9.

5 Scherpereel A, Astoul P, Baas P, *et al.* Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. Eur. Respir. J. 2010;**35**:479–95. doi:10.1183/09031936.00063109

6 Rusch VW, Giroux D. Do we need a revised staging system for malignant pleural mesothelioma? Analysis of the IASLC database. *Ann Cardiothorac Surg* 2012;**1**:438–48. doi:10.3978/j.issn.2225-319X.2012.11.10

7 Boutin C, Rey F. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: Diagnosis. *Cancer* 1993;**72**:389–93.

8 Salyer WR, Eggleston JC, Erozan YS. Efficacy of pleural needle biopsy and pleural fluid cytopathology in the diagnosis of malignant neoplasm involving the pleura. *Chest* 1975;**67**:536–9.

9 Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic

analysis for the evaluation of pleural effusion: analysis of 414 cases. *Mayo Clin Proc* 1985;**60**:158–64.

10 Nance KV, Shermer RW, Askin FB. Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid examination. *Mod Pathol* 1991;**4**:320–4.

11 O'Rourke N, Garcia JC, Paul J, *et al.* A randomised controlled trial of intervention site radiotherapy in malignant pleural mesothelioma. *Radiother Oncol* 2007;**84**:18–22. doi:10.1016/j.radonc.2007.05.022

12 Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. *Chest* 1995;**108**:754–8.

13 Robinson BW, Creaney J, Lake R, *et al.* Mesothelin-family proteins and diagnosis of mesothelioma. *The Lancet* 2003;**362**:1612–6. doi:10.1016/S0140-6736(03)14794-0

14 Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax* 2014;**69**:895–902. doi:10.1136/thoraxjnl-2014-205205

15 Creaney J, Yeoman D, Demelker Y, *et al.* Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. *J Thorac Oncol* 2008;**3**:851–7. doi:10.1097/JTO.0b013e318180477b

16 Pass HI, Lott D, Lonardo F, *et al.* Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med* 2005;**353**:1564–73. doi:10.1056/NEJMoa051185

17 Chang K, Pai LH, Batra JK, *et al.* Characterization of the antigen (CAK1) recognized by monoclonal antibody K1 present on ovarian cancers and normal mesothelium. *Cancer Res* 1992;**52**:181–6.

18 Chang K, Pai LH, Pass H, *et al.* Monoclonal antibody K1 reacts with

epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg Pathol* 1992;**16**:259–68.

19 Hollevoet K, Nackaerts K, Thimpont J, *et al.* Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. *Am J Respir Crit Care Med* 2010;**181**:620–5. doi:10.1164/rccm.200907-1020OC

20 Hooper C, Lee YCG, Maskell N, *et al.* Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax. 2010;**65 Suppl 2**:ii4–17. doi:10.1136/thx.2010.136978

21 Hollevoet K, Nackaerts K, Thas O, *et al.* The effect of clinical covariates on the diagnostic and prognostic value of soluble mesothelin and megakaryocyte potentiating factor. *Chest* 2012;**141**:477–84. doi:10.1378/chest.11-0129

22 Bergamini S, Bellei E, Bonetti LR, *et al.* Inflammation: an important parameter in the search of prostate cancer biomarkers. *Proteome Science 2014 12:1* 2014;**12**:32. doi:10.1186/1477-5956-12-32

23 Yuan JJ, Coplen DE, Petros JA, *et al.* Effects of rectal examination, prostatic massage, ultrasonography and needle biopsy on serum prostate specific antigen levels. *J Urol* 1992;**147**:810–4.

24 Wong V, Wang D-Y, Warren K, *et al.* The effects of timing of fine needle aspiration biopsies on gene expression profiles in breast cancers. *BMC Cancer* 2008;**8**:277. doi:10.1186/1471-2407-8-277

25 Pass HI, Levin SM, Harbut MR, *et al.* Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *N Engl J Med* 2012;**367**:1417–27. doi:10.1056/NEJMoa1115050

26 Kirschner MB, Pulford E, Hoda MA, *et al.* Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. *Br J Cancer* 2015;**113**:963–9. doi:10.1038/bjc.2015.286

27 Ostroff RM, Mehan MR, Stewart A, *et al.* Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. *PLoS ONE* 2012;**7**:e46091. doi:10.1371/journal.pone.0046091

28 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004. doi:10.1371/journal.pone.0015004

29 Kao SCH, Pavlakis N, Harvie R, *et al.* High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. *Clin Cancer Res* 2010;**16**:5805–13. doi:10.1158/1078-0432.CCR-10-2245

30 Hooper CE, Lyburn ID, Searle J, *et al.* The South West Area Mesothelioma and Pemetrexed trial: a multicentre prospective observational study evaluating novel markers of chemotherapy response and prognostication. *Br J Cancer* 2015;**112**:1175–82. doi:10.1038/bjc.2015.62

31 Pinato DJ, Mauri FA, Ramakrishnan R, *et al.* Inflammation-Based Prognostic Indices in Malignant Pleural Mesothelioma. *Journal of Thoracic Oncology* 2012;**7**:587–94. doi:10.1097/JTO.0b013e31823f45c1

32 Burt BM, Rodig SJ, Tilleman TR, *et al.* Circulating and tumor-infiltrating myeloid cells predict survival in human pleural mesothelioma. *Cancer* 2011;**117**:5234–44. doi:10.1002/cncr.26143

33 Zhang Y, Marmorstein LY. Focus on molecules: Fibulin-3 (EFEMP1). *Experimental Eye Research* 2010;**90**:374–5. doi:10.1016/j.exer.2009.09.018

34 Agha MA, El-Habashy MM, El-Shazly RA. Role of fibulin-3 in the diagnosis of malignant mesothelioma. *Egyptian Journal of Chest Diseases and Tuberculosis* 2014;**63**:99–105. doi:10.1016/j.ejcdt.2013.10.004

35 Corradi M, Goldoni M, Alinovi R, *et al.* YKL-40 and mesothelin in the blood of patients with malignant mesothelioma, lung cancer and asbestosis. *Anticancer Res* 2013;**33**:5517–24.

36 Creaney J, Francis RJ, Dick IM, *et al.* Serum Soluble Mesothelin Concentrations in Malignant Pleural Mesothelioma: Relationship to Tumor Volume, Clinical Stage and Changes in Tumor Burden. *Clin Cancer Res* 2011;**17**:1181–9. doi:10.1158/1078-0432.CCR-10-1929

37 Pairon J-C, Laurent F, Rinaldo M, *et al.* Pleural plaques and the risk of pleural mesothelioma. *J Natl Cancer Inst* 2013;**105**:293–301. doi:10.1093/jnci/djs513

38 Paris C, Thierry S, Brochard P, *et al.* Pleural plaques and asbestosis: dose- and time-response relationships based on HRCT data. *Eur Respir J* 2009;**34**:72–9. doi:10.1183/09031936.00094008

39 Baldwin DR, Callister MEJ, Guideline Development Group. The British Thoracic Society guidelines on the investigation and management of pulmonary nodules. *Thorax* 2015;**70**:794–8. doi:10.1136/thoraxjnl-2015-207221

40 HSE. The Asbestos Survey. http://www.hse.gov.uk 2016;:1–150.

41 Napolitano A, Antoine DJ, Pellegrini L, *et al.* HMGB1 and Its Hyperacetylated Isoform are Sensitive and Specific Serum Biomarkers to Detect Asbestos Exposure and to Identify Mesothelioma Patients. *Clin Cancer Res* Published Online First: 5 January 2016. doi:10.1158/1078-0432.CCR-15-1130

42 Services UDOHAH. *Guidance for Industry Bioanalytical Method Validation (2001) http://www. fda. gov/downloads/Drugs/GuidanceComplianceRegulator y Information*.

43 Frauenfelder T, Tutic M, Weder W, *et al.* Volumetry: an alternative to assess therapy response for malignant pleural mesothelioma? *Eur Respir J* 2011;**38**:162–8. doi:10.1183/09031936.00146110

## CONTRIBUTIONS

Selina Tsim

- Contribution to the conception or design of the work; data acquisition, analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Caroline Kelly

- Contribution to the design of the work; analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Laura Alexander

- Contribution to the design of the work and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that

questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Carol McCormick

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Fiona Thomson

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Rosie Woodward

- Contribution to the design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content

- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

John E Foster

- Contribution to the conception and design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

David B Stobo

- Contribution to the design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Jim Paul

- Contribution to the conception and design of the work; data analysis

or interpretation of data for the work

- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Nick A Maskell

- Contribution to the design of the work; data analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Anthony Chalmers

- Contribution to the conception and design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kevin G Blyth

- Principal contribution to the conception and design of the work; data acquisition, analysis and interpretation of data for the work
- Drafting the work
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

**FUNDING**

This work was supported by the Chief Scientist's Office of the Scottish Government (Project Grant ETM/285) and the West of Scotland Lung Cancer Research Group (Award September 2015). KGB is part-funded by NHS Research Scotland.

**COMPETING INTERESTS STATEMENT**

SomaLogic Inc. have provided funding for all SOMAscan assays.

## FIGURE LEGENDS

### Figure 1

Summary of the design of the DIAPHRAGM study. Figure 1(a) is intended to describe the optimal diagnostic pathway for the majority of patients who present with significant Pleural Effusion +/- pleural thickening or a pleural mass. Figure 1(b) describes the optimal diagnostic pathway for the minority of patients who present with an isolated Pleural Mass, but no significant fluid component. The pathway chosen is ultimately at the discretion of the investigating physician.

## TABLES

**Table 1. Outcome Measures used in the DIAPHRAGM study**

| Research Objective | Outcome Measures |
|---|---|
| **Primary**<br>To determine whether SOMAscan results and/or Fibulin-3 levels in blood at presentation can differentiate MPM from asbestos-exposed controls and patients with other causes of pleural effusion with a sufficient degree of sensitivity and specificity to be of routine clinical value | Serum SOMAscan<br>Plasma Fibulin-3<br>Final diagnosis reached |
| **Secondary**<br>To determine whether:<br>1. SOMAscan results and/or Fibulin-3 levels at presentation provide clinically useful prognostic information in MPM patients | Serum SOMAscan & plasma Fibulin-3 at presentation<br>Survival (from registration) |
| 2. early changes in SOMAscan and/or Fibulin-3 levels after diagnosis (at 3 months) are associated with a poorer prognosis in MPM | Serum SOMAscan & plasma Fibulin-3 3 months post-Dx<br>Survival (from registration) |
| **Exploratory**<br>To determine whether:<br>1. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour volume, defined by MRI | Serum SOMAscan<br>Plasma Fibulin-3<br>MPM tumour volume at MRI, defined using Myrian intrasense™ software |
| 2. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour angiogenesis (as defined by perfusion-based MRI biomarkers) | Serum SOMAscan<br>Plasma Fibulin-3<br>The following MRI biomarkers:<br>• MRI-ECE<br>• Redistribution rate contstant ($K_{ep}$)<br>• Elimination rate constant ($K_{el}$) |
| 3. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and pleural fluid at presentation in patients with MPM | SOMAscan and Fibulin-3 at presentation and at 1 month post-biopsy +/- drainage and pleurodesis |

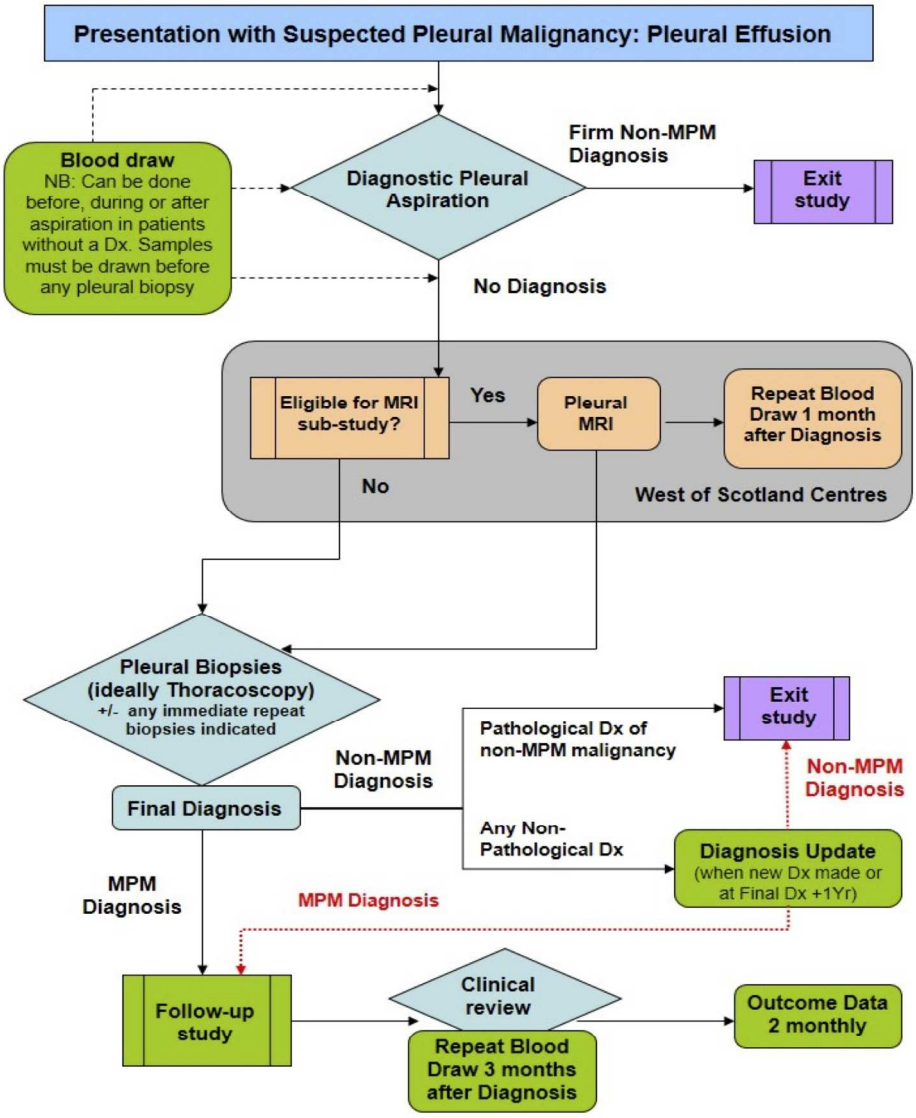



Figure 1(a)

156x180mm (300 x 300 DPI)

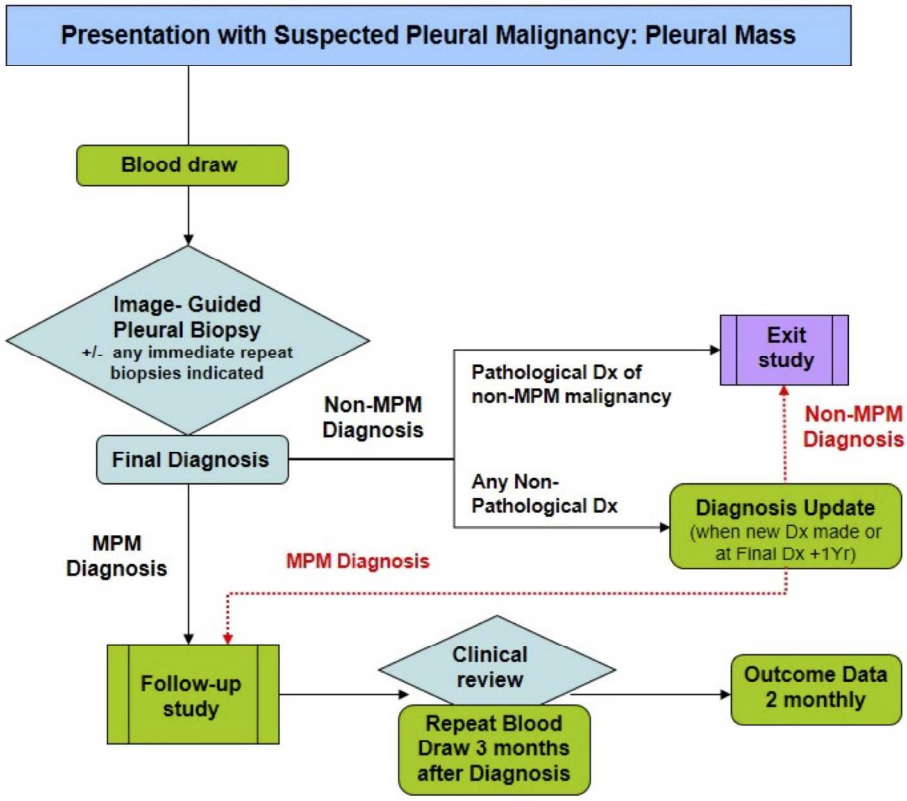



Figure 1(b)

166x144mm (300 x 300 DPI)

CANCER RESEARCH UK
Clinical Trials Unit, Glasgow

# ASBESTOS EXPOSURE QUESTIONNAIRE

(to be completed for all patients on study)

## DIAPHRAGM: Diagnostic and Prognostic biomarkers in the Rational Assessment of Mesothelioma

| | |
|---|---|
| **PATIENT INITIALS:** (f) _____ (s) _____ | **DATE of BIRTH:** DD / MON / YYYY |
| **INVESTIGATOR:** | **REGISTRATION DATE:** DD / MON / YYYY |
| **SITE:** | **PATIENT TRIAL IDENTIFIER:** |
| **DATE OF COMPLETION OF QUESTIONNAIRE:** DD / MON / YYYY | |

**Employment History** **No known asbestos exposure ☐**

| Industry / Occupation e.g. joiner, shipyard worker | Period of Employment: Start Year | Period of Employment: No. of Years | Period of Employment: No. of Months | No. of Hours per day | Days per week | Airway protection? | Job Type Please refer to codes below | Job Code Please refer to Page 2 | Indirect Exposure Details e.g. via husband, via father |
|---|---|---|---|---|---|---|---|---|---|
| **1.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **2.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **3.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **4.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |
| **5.** | YYYY | | | _____ | _____ | Yes ☐ No ☐ | | | |

**JOB TYPE:** **M** = Manufacturing asbestos products **S** = Asbestos Stripping/Removal **O** = Something else **I** = Indirect exposure

**DATE:** DD / MON / YYYY **INVESTIGATOR'S SIGNATURE**: _____

# Job Codes
## (Taken from Appendix 5 of the HSE Asbestos Survey)

### CODE LIST M

**Textile manufacture**
01 Raw material store
02 Raw material & finished product transport
03 Disintegrating/heating/opening/fibrising
04 Hopper feeding
05 Carding
06 Weaving
07 Spinning
08 Doubling/twisting
09 Braiding
10 Warping
11 Detritus handling
12 Inspecting
13 Supervising
14 Finished product store & dispatch
15 Other exposed workers
95 'Fortex' process

**Asbestos cement mixture board and pipe manufacture**
16 Supervising
17 Raw material store
18 Raw material transport
19 Disintegrating
20 Mixing/beating
21 Wet board or pipe manufacture
22 Wet board or pipe handling
23 Drying
24 Dry board handling
25 Machining or cutting
26 Sanding
27 Inspecting
28 Finished product store/packing/dispatch/transport
29 Detritus handling wet
30 Detritus handling dry
31 Other exposed worker

**Manufacture of asbestos/rubber/resin bitumen mixtures**
32 Raw material store
33 Transporting raw materials
34 Disintegrating
35 Handling raw fibre (bag tipping/weighting/mixing)
36 Pressing/moulding
37 Cutting/finishing/machining
38 Transporting finished product
39 Finished product storage/packing/dispatching
40 Inspecting
41 Supervising
42 Other exposed worker

**Asbestos board and paper manufacture**
43 Raw material store
44 Raw material transport
45 Disintegrating/opening/fibrising
46 Mixing/beating
47 Handling wet mixture
48 Drying
49 Handling dry mixture
50 Cutting/machining
51 Store/transport/packing/dispatching products
52 Supervising
53 Detritus handling
54 Inspecting
55 Other exposed workers

**Garment manufacture**
56 Cloth store
57 Cutting out
58 Stitching
59 Transport of materials
60 Storing/packing/dispatching
61 Inspecting
62 Supervising
63 Other exposed workers

**Manufacture of dry mixes for insulation & plastering**
70 Raw material stores
71 Raw material handling/bag tipping/weighing/mixing
72 Packaging
73 Stores and dispatch
74 Other exposed workers

**Maintenance workers all manufacturing sectors**
75 Supervising
76 Fault finder/machine fitter/installation engineer/plant engineer
77 Labour to plant engineers etc
78 Carpenters/joiners
79 Electrician
80 Plumber
81 Other building trade craftsmen eg painter
82 Labourer to building trade craftsman
83 Ventilation plant servicing
84 Factory cleaning

### CODE LIST S

**Asbestos stripping/removal**
64 Supervising
65 Stripping/encapsulating
69 Other exposed workers (eg sampler, cleaner, scaffolder)

### CODE LIST O

**Ship building, repair, & breaking**
85 Asbestos storeman
86 Lagging
87 Boilermakers and installers
88 Carpenters/joiners
89 Plumber
90 Engine fitter
91 Other exposed workers
92 Asbestos stripping
93 Cleaner
94 Shipbreaking

**Building & construction**
96 Heating engineer
97 Asbestos board cutting/fitting
98 Asbestos roofing construction and maintenance
99 Demolition
104 Other building trade craftsmen eg painter *(81)*†
105 Labourer to building trade craftsman *(82)*
106 Plumber *(80)*
107 Carpenters/joiners *(78)*

**Miscellaneous processes**
101 Use of asbestos string/rope/felt *(65)*
102 Fitting clutch and brake pads *(37)*
108 Machining/cutting asbestos/resin board *(50)*
109 Other exposed workers *(69)*